Kv6.2, A VOLTAGE-GATED POTASSIUM CHANNEL SUBUNIT

CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims the benefit of USSN 60/091,466, filed July 1, 1998, herein incorporated by reference in its entirety.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

Not applicable.

FIELD OF THE INVENTION

The invention provides isolated nucleic acid and amino acid sequences of Kv6.2, antibodies to Kv6.2, methods of detecting Kv6.2, methods of screening for voltage-gated potassium channel activators and inhibitors using biologically active Kv6.2, and kits for screening for activators and inhibitors of voltage gated potassium channels comprising Kv6.2.

BACKGROUND OF THE INVENTION

Potassium channels are involved in a number of physiological processes, including regulation of heartbeat, dilation of arteries, release of insulin, excitability of nerve cells, and regulation of renal electrolyte transport. Potassium channels are thus found in a wide variety of animal cells such as nervous, muscular, glandular, immune, reproductive, and epithelial tissue. These channels allow the flow of potassium in and/or out of the cell under certain conditions. For example, the outward flow of potassium ions upon opening of these channels makes the interior of the cell more negative, counteracting depolarizing voltages applied to the cell. These channels are regulated, e.g., by calcium sensitivity, voltage-gating, second messengers, extracellular ligands, and ATP-sensitivity.

Potassium channels are made by alpha subunits that fall into 8 families, based on predicted structural and functional similarities (Wei *et al.*, *Neuropharmacology* 35(7):805-829 (1997)). Three of these families (Kv, Eag-related, and KQT) share a common motif of six transmembrane domains and are primarily gated by voltage. Two

5 other families, CNG and SK/IK, also contain this motif but are gated by cyclic nucleotides and calcium, respectively. The three other families of potassium channel alpha subunits have distinct patterns of transmembrane domains. Slo family potassium channels, or BK channels have seven transmembrane domains (Meera *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 94(25):14066-71 (1997)) and are gated by both voltage and calcium or

10 pH (Schreiber *et al.*, *J. Biol. Chem.* 273:3509-16 (1998)). Another family, the inward rectifier potassium channels (Kir), belong to a structural family containing 2 transmembrane domains (*see, e.g.*, Lagrutta *et al.*, *Jpn. Heart. J.* 37:651-660 1996)), and an eighth functionally diverse family (TP, or "two-pore") contains 2 tandem repeats of this inward rectifier motif.

15 Potassium channels are typically formed by four alpha subunits, and can be homomeric (made of identical alpha subunits) or heteromeric (made of two or more distinct types of alpha subunits). In addition, potassium channels made from Kv, KQT and Slo or BK subunits have often been found to contain additional, structurally distinct auxiliary, or beta, subunits. These beta subunits do not form potassium channels

20 themselves, but instead they act as auxiliary subunits to modify the functional properties of channels formed by alpha subunits. For example, the Kv beta subunits are cytoplasmic and are known to increase the surface expression of Kv channels and/or modify inactivation kinetics of the channel (Heinemann *et al.*, *J. Physiol.* 493:625-633 (1996); Shi *et al.*, *Neuron* 16(4):843-852 (1996)). In another example, the KQT family beta

25 subunit, minK, primarily changes activation kinetics (Sanguinetti *et al.*, *Nature* 384:80-83 (1996)).

The Kv superfamily of voltage-gated potassium channels includes both heteromeric and homomeric channels that are typically composed of four subunits (*see, e.g.*, Salinas *et al.*, *J. Biol. Chem.* 272:8774-8780 (1997); Salinas *et al.*, *J. Biol. Chem*

30 272:24371-24379 (1997); Post *et al.*, *FEBS Letts.* 399:177-182 (1996)). Voltage-gated potassium channels have been found in a wide variety of tissues and cell types and are

involved in processes such as neuronal integration, cardiac pacemaking, muscle contraction, hormone section, cell volume regulation, lymphocyte differentiation, and cell proliferation (*see, e.g.*, Salinas *et al., J. Biol. Chem.* 39:24371-24379 (1997)). Some alpha subunits of the Kv superfamily, of which the channels are composed, have

5 been cloned and expressed, e.g., Kv5.1, Kv6.1 (Drewe *et al., J. Neurosci.* 12:538-548 (1992); Post *et al., FEBS Letts.* 399:177-182 (1996)); Kv8.1 (Hugnot *et al., EMBO J.* 15:3322-3331 (1996)); and Kv9.1 and 9.2 (Salinas *et al., J. Biol. Chem.* 39:24371-24379 (1997)). Expression patterns of some of these genes has also been examined (*see, e.g.*, Verma-Kurvari *et al., Mol. Brain. Res.* 46:54-62 (1997); Maletic-Savatic *et*

10 *al., J. Neurosci.* 15:3840-3851 (1995); Du *et al., Neurosci.* 84:37-48 (1998)).

SUMMARY OF THE INVENTION

The present invention thus provides for the first time Kv6.2, a polypeptide monomer that is an alpha subunit of an heteromeric voltage-gated potassium

15 channel. Kv6.2 has not been previously cloned or identified, and the present invention provides the nucleotide and amino acid sequences for mouse and human Kv6.2.

In one aspect, the present invention provides an isolated nucleic acid encoding a polypeptide monomer comprising an alpha subunit of a heteromeric potassium channel, the polypeptide monomer: (i) having the ability to form, with at

20 least one additional Kv alpha subunit, a heteromeric potassium channel having the characteristic of voltage gating; (ii) having a monomer subunit association region that has greater than 70% amino acid sequence identity to a Kv6.2 subunit association region; and (iii) specifically binding to polyclonal antibodies generated against SEQ ID NO:1 or SEQ ID NO:17.

25 In one aspect, the present invention provides an isolated nucleic acid encoding a polypeptide monomer comprising an alpha subunit of a heteromeric potassium channel, the polypeptide monomer: (i) having the ability to form, with at least one additional Kv alpha subunit, a heteromeric potassium channel having the characteristic of voltage gating; (ii) having an S4-S6 region that has greater than 85%

amino acid sequence identity to a Kv6.2 S4-S6 region; and (iii) specifically binding to polyclonal antibodies generated against SEQ ID NO:1 or SEQ ID NO:17.

In one embodiment, the nucleic acid encodes mouse or human Kv6.2. In another embodiment, the nucleic acid encodes SEQ ID NO:1 or SEQ ID NO:17. In

5   another embodiment, the nucleic acid has a nucleotide sequence of SEQ ID NO:2 or SEQ ID NO:18.

In one embodiment, the nucleic acid is amplified by primers that selectively hybridize under stringent hybridization conditions to the same sequence as the primer sets selected from the group consisting of:

10   ATGCCCATGTCTTCCAGAGACAGG (SEQ ID NO:3),

GATGTCTAGAGGGAGTTACATGTAGCG (SEQ ID NO:4) and

GGCACTACGCATCCTCTACGTAATGCGC (SEQ ID NO:5),

GATGATGGCCCACCAATAGGATGCGG (SEQ ID NO:6) and

ATGCCCATGCCTTCCAGAGACGG (SEQ ID NO:7),

15   TTACATGTGCATGATAGGCAAGGCTG (SEQ ID NO:8) and

GTCCAGGCCCAAGACAAGTGTCAG (SEQ ID NO:9),

GGGAGAAGGTGTGGAAGATAGACG (SEQ ID NO:10).

In one embodiment, the nucleic acid encodes a polypeptide monomer having a molecular weight of between about 53 kDa to about 65 kDa. In one

20   embodiment, the nucleic acid selectively hybridizes under moderately stringent hybridization conditions to a nucleotide sequence of SEQ ID NO:2 or SEQ ID NO:18.

In another aspect, the present invention provides an isolated nucleic acid encoding a polypeptide monomer, wherein the nucleic acid specifically hybridizes under highly stringent conditions to SEQ ID NO:2 or SEQ ID NO:18.

25   In another aspect, the present invention provides an isolated polypeptide monomer comprising an alpha subunit of a heteromeric potassium channel, the potassium channel: (i) having the ability to form, with at least one additional Kv alpha subunit, a heteromeric potassium channel having the characteristic of voltage gating; (ii) having a monomer subunit association region that has greater than 70% amino acid

30   sequence identity to amino acids a Kv6.2 subunit association region; and (iv)

specifically binding to polyclonal antibodies generated against SEQ ID NO:1 or SEQ ID NO:17.

In another aspect, the present invention provides an isolated polypeptide monomer comprising an alpha subunit of a heteromeric potassium channel, the

5 potassium channel: (i) having the ability to form, with at least one additional Kv alpha subunit, a heteromeric potassium channel having the characteristic of voltage gating; (ii) having an S4-S6 region that has greater than 85% amino acid sequence identity to a Kv6.2 S4-S6 region; and (iv) specifically binding to polyclonal antibodies generated against SEQ ID NO:1 or SEQ ID NO:17.

10 In one embodiment, the polypeptide monomer has an amino acid sequence of mouse or human Kv6.2. In another embodiment, the polypeptide monomer has an amino acid sequence of SEQ ID NO:1 or SEQ ID NO:17.

In another aspect, the present invention provides an antibody that selectively binds to the polypeptide monomer described above.

15 In another aspect, the present invention provides an expression vector comprising the nucleic acid encoding the polypeptide monomer described above.

In another aspect, the present invention provides a host cell transfected with the expression vector described above.

In another aspect, the present invention provides a method for identifying

20 a compound that increases or decreases ion flux through an voltage-gated potassium channel, the method comprising the steps of: (i) contacting the compound with a eukaryotic host cell or cell membrane in which has been expressed a polypeptide monomer comprising an alpha subunit of a heteromeric potassium channel, the polypeptide monomer: (a) having the ability to form, with at least one additional Kv

25 alpha subunit, a heteromeric potassium channel having the characteristic of voltage gating; (b) having a monomer subunit association region that has greater than 70% amino acid sequence identity to a Kv6.2 subunit association region; and (c) specifically binding to polyclonal antibodies generated against SEQ ID NO:1 or SEQ ID NO:17; and (ii) determining the functional effect of the compound upon the cell or cell

30 membrane expressing the potassium channel.

In another aspect, the present invention provides a method for identifying a compound that increases or decreases ion flux through an voltage-gated potassium channel, the method comprising the steps of: (i) contacting the compound with a eukaryotic host cell or cell membrane in which has been expressed a polypeptide

5   monomer comprising an alpha subunit of a heteromeric potassium channel, the polypeptide monomer: (a) having the ability to form, with at least one additional Kv alpha subunit, a heteromeric potassium channel having the characteristic of voltage gating; (b) having an S4-S6 region that has greater than 85% amino acid sequence identity to a Kv6.2 S4-S6 region as measured using a sequence comparison algorithm;

10   and (c) specifically binding to polyclonal antibodies generated against SEQ ID NO:1 or SEQ ID NO:17; and (ii) determining the functional effect of the compound upon the cell or cell membrane expressing the potassium channel.

In one embodiment, the increased or decreased flux of ions is determined by measuring changes in current or voltage. In another embodiment, the polypeptide

15   monomer polypeptide is recombinant.

In another embodiment, the present invention provides a method of detecting the presence of Kv6.2 in mammalian tissue, the method comprising the steps of: (i) isolating a biological sample; (ii) contacting the biological sample with a Kv6.2-specific reagent that selectively associates with Kv6.2; and, (iii) detecting the level of

20   Kv6.2-specific reagent that selectively associates with the sample.

In one embodiment, the Kv6.2-specific reagent is selected from the group consisting of: Kv6.2 specific antibodies, Kv6.2 specific oligonucleotide primers, and Kv6.2 nucleic acid probes. In another embodiment, the sample is from a human.

In another aspect, the present invention provides, in a computer system,

25   a method of screening for mutations of Kv6.2 genes, the method comprising the steps of: (i) entering into the computer a first nucleic acid sequence encoding an voltage-gated potassium channel protein having a nucleotide sequence of SEQ ID NO:2 or SEQ ID NO:18, and conservatively modified versions thereof; (ii) comparing the first nucleic acid sequence with a second nucleic acid sequence having substantial identity to the first

nucleic acid sequence; and (iii) identifying nucleotide differences between the first and second nucleic acid sequences.

In one embodiment, the second nucleic acid sequence is associated with a disease state.

5   In another aspect, the present invention provides, in a computer system, a method for identifying a three-dimensional structure of Kv6.2 polypeptides, the method comprising the steps of: (i) entering into the computer system an amino acid sequence of at least 25 amino acids of a potassium channel monomer or at least 75 nucleotides of a gene encoding the polypeptide, the polypeptide having an amino acid

10   sequence of SEQ ID NO:1 or SEQ ID NO:17, and conservatively modified versions thereof; and (ii) generating a three-dimensional structure of the polypeptide encoded by the amino acid sequence.

In one embodiment, the amino acid sequence is a primary structure and wherein said generating step includes the steps of: (i) forming a secondary structure

15   from said primary structure using energy terms determined by the primary structure; and (ii) forming a tertiary structure from said secondary structure using energy terms determined by said secondary structure. In another embodiment, the generating step includes the step of forming a quaternary structure from said tertiary structure using anisotropic terms determined by the tertiary structure. In another embodiment, the

20   methods further comprises the step of identifying regions of the three-dimensional structure of the protein that bind to ligands and using the regions to identify ligands that bind to the protein.

BRIEF DESCRIPTION OF THE DRAWINGS

25   **Figure 1**. Amino acid alignment of the human and mouse Kv6.2 genes. Identical residues are shaded and gaps in the alignment are indicated by dashes. Amino acid residue numbers are given at the left margin. Human and mouse Kv6.2 are 80% identical overall on the amino acid level.

30   **Figure 2**. Expression of Kv6.2 genes in Xenopus oocytes. (A) Currents recorded from an ooycte injected with cRNA from the mouse Kv6.2 gene. Voltage steps

used were from a resting potential of –90 mV and ranged from –80 mV to +20 mV in 20

mV increments. Neither mouse or human Kv6.2 gave rise to outward voltage-gated

potassium currents under these conditions. (B-D) Currents recorded from oocytes

injected with cRNA for the human Kv2.1 gene alone (B), for a coinjection with human

5 Kv2.1 and mouse Kv6.2 (C), and for a coinjection of human Kv2.1 and human Kv6.2.

Identical voltage steps were applied to each egg. .Resting potential in each case was –90

mV and the voltage steps ranged from –80 mV to +20 mV in 20 mV increments. Note

both Kv6.2/Kv2.1 heteromers activate at more hyperpolarized voltages than Kv2.1

homomers. Also note that the deactivation of the heteromers is much slower than that

10 seen for Kv2.1 homomers. The amount of Kv2.1 cRNA used in (B) was one eighth of

that used in (C) and (D). Both Kv6.2 genes consistently cause a reduction in the size of

the Kv2.1 current.

DETAILED DESCRIPTION OF THE INVENTION

15 **I.   INTRODUCTION**

The present invention provides for the first time a nucleic acid encoding

Kv6.2, identified and cloned from mouse and human tissue. This polypeptide monomer

is a member of the "Kv" superfamily of potassium channel monomers. Members of

this family are polypeptide monomers that are subunits of voltage-gated potassium

20 channels having six transmembrane regions (K=potassium, v=voltage-gated).

Voltage-gated potassium channels have significant roles in maintaining the resting

potential and in controlling excitability of a cell.

The invention also provides methods of screening for activators and

inhibitors of voltage-gated potassium channels that contain a Kv6.2 subunit. Such

25 modulators of voltage-gated channel activity are useful for treating CNS disorders such

as migraines, hearing and vision problems, psychotic disorders, seizures, and as

neuroprotective agents (e.g., to prevent stroke).

Furthermore, the invention provides assays f : Kv6.2 activity where

Kv6.2 acts as a direct or indirect reporter molecule. Such uses of Kv6.2 as a reporter

30 molecule in assay and detection systems have broad applications, e.g., Kv6.2 can be

used as a reporter molecule to measure changes in potassium concentration, membrane

potential, current flow, ion flux, transcription, signal transduction, receptor-ligand interactions, second messenger concentrations, *in vitro*, *in vivo*, and *ex vivo*. In one embodiment, Kv6.2 can be used as an indicator of current flow in a particular direction (e.g., outward or inward potassium flow), and in another embodiment, Kv6.2 can be

5 used as an indirect reporter via attachment to a second reporter molecule such as green fluorescent protein.

Finally, the invention provides for methods of detecting Kv6.2 nucleic acid and protein expression, allowing investigation of the channel diversity provided by Kv6.2 and the regulation/modulation of heteromeric channel activity provided by

10 Kv6.2, as well as diagnosis of disease involving abnormal ion flux, including diagnosis of CNS disease such as migraines, hearing and vision problems, seizures and psychotic disorders.

Functionally, Kv6.2 is an alpha subunit of an voltage-gated potassium channel. Typically, such voltage-gated channels are heteromeric or homomeric and

15 contain four subunits or monomers each with six transmembrane domains. Voltage-gated potassium channels comprising Kv6.2 are typically heteromeric and may contain one or more subunits of Kv6.2 along with one or more other subunits from the Kv superfamily, e.g., Kv 2.1 and Kv 2.2. The presence of Kv6.2 in an voltage-gated potassium channel modulates the activity of the heteromeric channel and thus enhances

20 channel diversity. For example, when Kv6.2 associates with another monomer, the resulting channel may have a distinct single channel conductance as well as altered kinetic properties, e.g., changes in activation or inactivation rates and changes in voltages and thresholds for activation. For example, Figure 2 shows a hyperpolarized shift in activation voltages by about 20 mV, and a dramatic slowing of deactivation in

25 channels comprising Kv6.2 and Kv2.1 as compared to channels comprising only Kv2.1. Channel diversity is also enhanced with alternatively spliced forms of Kv6.2.

Structurally, the nucleotide sequence of mouse Kv6.2 (SEQ ID NO:2) encodes a polypeptide monomer of approximately 506 amino acids with a predicted molecular weight of approximately 58 kDa (SEQ ID NO:1) and a predicted range of 53-

30 63 kDa. The nucleotide sequence of human Kv6.2 (SEQ ID NO:18) encodes a

polypeptide monomer of approximately 519 amino acids with a predicted molecular weight of approximately 60 kDa (SEQ ID NO:17) and a predicted range of 55-65 kDa. In particular, the amino acid sequence of Kv6.2 has a "subunit association" region (approximately amino acids 70 to 182, *see, e.g.*, amino acids 70-182 of SEQ ID NO:1,

5   mouse Kv6.2) that has a conserved amino acid sequence. Related Kv6.2 genes from other species and/or Kv6 family members share at least about 70% amino acid identity in this region. The amino acid sequence of Kv6.2 also has a conserved S4-S6 region (approximately amino acids 326-466, *see, e.g.*, amino acids 326-466 of SEQ ID NO 1, mouse Kv6.2). Related Kv6.2 genes from other species and/or Kv6 family members

10   share at least about 85% amino acid identity in this region.

The present invention also provide polymorphic variants of the Kv6.2 depicted in SEQ ID NO:1: variant #1, in which a aspartate residue is substituted for the glutamate residue at amino acid position 484; variant #2, in which a valine residue is substituted for the leucine residue at amino acid position 174; and variant #3, in which a

15   serine residue is substituted for the alanine residue at amino acid position 195.

The present invention also provide polymorphic variants of the Kv6.2 depicted in SEQ ID NO:17: variant #1, in which a leucine residue is substituted for the methionine residue at amino acid position 501; variant #2, in which a serine residue is substituted for the alanine residue at amino acid position 148; variant #3, in which a

20   valine residue is substituted for the isoleucine residue at amino acid position 508; and variant #4, in which a phenylalanine residue is substituted for the tyrosine residue at amino acid position 17.

Specific regions of the Kv6.2 nucleotide and amino acid sequence may be used to identify polymorphic variants, interspecies homologs, and alleles of Kv6.2.

25   This identification can be made *in vitro*, e.g., under stringent hybridization conditions and sequencing, or by using the sequence information in a computer system for comparison with other nucleotide sequences, or using antibodies raised against Kv6.2. Typically, identification of polymorphic variants and alleles of Kv6.2 is made by comparing the amino acid sequence (or the nucleic acid sequenc encoding the amino

30   acid sequence) of the subunit association region (approximately amino acids 70-182 of

mouse Kv6.2, see SEQ ID NO:1 for example) or the S4-S6 region (approximately amino acids 326-466 of mouse Kv6.2, see SEQ ID NO:1 for example). Amino acid identity of approximately at least 70% or above, preferably 80 %, 85%, most preferably 90-95% or above in the subunit association region or the S4-S6 region

5   typically demonstrates that a protein is a polymorphic variant, interspecies homolog, or allele of Kv6.2. Sequence comparison can be performed using any of the sequence comparison algorithms discussed below. Antibodies that bind specifically to the subunit association region of Kv6.2 can also be used to identify alleles, interspecies homologs, and polymorphic variants.

10   Polymorphic variants, interspecies homologs, and alleles of Kv6.2 are confirmed by co-expressing the putative Kv6.2 polypeptide monomer and examining whether the monomer forms a heteromeric voltage-gated potassium channel, when co-expressed with another member of the Kv family such as Kv 2.1 or 2.2. This assay is used to demonstrate that a protein having about 70% or greater, preferably 75, 80, 85,

15   90, or 95% or greater amino acid identity to the "subunit association" region of Kv6.2 shares the same functional characteristics as Kv6.2 and is therefore a species of Kv6.2. This assay is also used to demonstrate that a protein having about 85% or greater, preferably 90%, 95% or greater amino acid identity to the "S4-S6" region of Kv6.2 shares the same functional characteristics as Kv6.2 and is therefore a species of Kv6.2.

20   Typically, Kv6.2 having the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:17 is used as a positive control in comparison to the putative Kv6.2 protein to demonstrate the identification of a polymorphic variant or allele of Kv6.2.

             Kv6.2 nucleotide and amino acid sequence information may also be used to construct models of a heteromeric voltage-gated potassium channels in a computer

25   system. These models are subsequently used to identify compounds that can activate or inhibit heteromeric voltage-gated potassium channels comprising Kv6.2. Such compounds that modulate the activity of channels comprising Kv6.2 can be used to investigate the role of Kv6.2 in modulation of channel activity and in channel diversity.

             The isolation of biologically active Kv6.2 for the first time provides a

30   means for assaying for inhibitors and activators of heteromeric voltage-gated potassium

channels that comprise Kv6.2 subunits. Biologically active Kv6.2 is useful for testing inhibitors and activators of voltage-gated potassium channels comprising subunits of Kv6.2 and other Kv members using *in vivo* and *in vitro* expression that measure, e.g., changes in voltage or current. Such activators and inhibitors identified using an

5   voltage-gated potassium channel comprising at least one Kv6.2 subunit can be used to further study voltage gating, channel kinetics and conductance properties of heteromeric channels. Such activators and inhibitors are useful as pharmaceutical agents for treating diseases involving abnormal ion flux, e.g., CNS disorders, as described above. Methods of detecting Kv6.2 and expression of channels comprising Kv6.2 are also

10   useful for diagnostic applications for diseases involving abnormal ion flux, e.g., CNS disorders and other disorders. For example, chromosome localization of the gene encoding Kv6.2 can be used to identify diseases caused by and associated with Kv6.2. Methods of detecting Kv6.2 are also useful for examining the role of Kv6.2 in channel diversity and modulation of channel activity.

15

II.   DEFINITIONS

As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

The phrase "voltage-gated" activity or "voltage-gating" refers to a

20   characteristic of a potassium channel composed of individual polypeptide monomers or subunits. Generally, the probability of a voltage-gated potassium channel opening increases as a cell is depolarized. Voltage-gated potassium channels primarily allow efflux of potassium because they have greater probabilities of being open at membrane potentials more positive than the membrane potential for potassium (E_K) in typical cells.

25   E_K, or the membrane potential for potassium, depends on the relative concentrations of potassium found inside and outside the cell membrane, and is typically between -60 and -100 mV for mammalian cells. E_K is the membrane potential at which there is no net flow of potassium ion because the electrical potential (i.e., voltage potential) driving potassium influx is balanced by the concentration gradient (the $[K^+]$ potential) directing potassium

30   efflux. This value is also known as the "reversal potential" or the "Nernst" potential for potassium. Some voltage-gated potassium channels undergo inactivation, which can

reduce potassium efflux at higher membrane potentials. Potassium channels can also allow potassium influx in certain instances when they remain open at membrane potentials negative to E_K (*see, e.g.,* Adams & Nonner, *in Potassium Channels*, pp. 40-60 (Cook, ed., 1990)). The characteristic of voltage gating can be measured by a

5   variety of techniques for measuring changes in current flow and ion flux through a channel, e.g., by changing the $[K^+]$ of the external solution and measuring the activation potential of the channel current (*see, e.g.,* U.S. Patent No. 5,670,335), by measuring current with patch clamp techniques or voltage clamp under different conditions, and by measuring ion flux with radiolabeled tracers or voltage-sensitive dyes

10   under different conditions.

"Homomeric channel" refers to an Kv6.2 channel composed of identical alpha subunits, whereas "heteromeric channel" refers to an Kv6.2 channel composed of at least one Kv alpha subunit plus at least one other different type of alpha subunit from the Kv family, e.g., Kv2.1. Both homomeric and heteromeric channels can include auxiliary

15   beta subunits. Typically, the channel is composed of four alpha subunits and the channel can be heteromeric or homomeric.

A "beta subunit" is a polypeptide monomer that is an auxiliary subunit of a potassium channel composed of alpha subunits; however, beta subunits alone cannot form a channel (*see, e.g.,* U.S. Patent No. 5,776,734). Beta subunits are known, for example,

20   to increase the number of channels by helping the alpha subunits reach the cell surface, change activation kinetics, and change the sensitivity of natural ligands binding to the channels. Beta subunits can be outside of the pore region and associated with alpha subunits comprising the pore region. They can also contribute to the external mouth of the pore region.

25   The phrase "subunit association region" refers to the region of Kv6.2 that structurally identifies this particular protein (approximately amino acids 70-182 of mouse Kv6.2, see SEQ ID NO:1). This region can be used to identify Kv6.2 polymorphic variants and Kv6.2 alleles of Kv6.2, through amino acid sequence identity comparison using a sequence comparison algorithm such as PILEUP. The subunit

30   association region is described in Shen *et al., Neuron* 11:67-76 (1993); Yu *et al., Neuron* 16:441-453 (1996); Xu *et al., J. Biol. Chem.* 270:24761-24768 (1995); Shen &

Pfaffinger, *Neuron* 14:625-633 (1995); Kreusch *et al., Nature* 392:945-948 (1998); and Li *et al., Science* 257:1225-1230 (1992).

The phrase "S4-S6 region" (S4 to S6 region) refers to the region of Kv6.2 that structurally identifies this particular protein (approximately amino acids 326-

5   466 of mouse Kv6.2, see SEQ ID NO:1). This region can be used to identify Kv6.2 polymorphic variants and Kv6.2 alleles of Kv6.2; through amino acid sequence identity comparison using a sequence comparison algorithm such as PILEUP. S4-S6 comprises three transmembrane regions: S4, S5, the pore domain, and S6 and is involved in voltage-gating and ion conduction (*see, e.g.*, Ackerman & Clapham, *New Engl. J. Med.*

10   336:1575-1586 (1997); Jan & Jan, *Annu. Rev. Neurosci.* 20:91-123 (1997)).

"Kv6.2" refers to a polypeptide that is a subunit or monomer of an voltage-gated potassium channel, a member of the Kv6 family, and a member of the Kv superfamily of potassium channel monomers. When Kv6.2 is part of a potassium channel, preferably a heteromeric potassium channel, the channel has voltage-gated

15   activity. The term Kv6.2 therefore refers to polymorphic variants, alleles, mutants, and interspecies homologs that: (1) have a subunit association region that has greater than about 70% amino acid sequence identity, preferably about 75, 80, 85, 90 or 95% amino acid sequence identity, to a Kv6.2 subunit association region; (2) have an S4-S6 region that has greater than about 85% amino acid sequence identity, preferably about 90 or

20   95% amino acid sequence identity, to a Kv6.2 S4-S6 region (3) bind to antibodies raised against an immunogen comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:17, the subunit association region, the S4-S6 region, and conservatively modified variants thereof; (4) specifically hybridize under stringent hybridization conditions to a sequence selected from the group consisting of

25   SEQ ID NO:2, SEQ ID NO:18, a nucleic acid encoding the subunit association region, a nucleic acid encoding the S4-S6 region, and conservatively modified variants thereof; or (5) are amplified by primers that specifically hybridize under stringent hybridization conditions to the same sequence as a primer set consisting of SEQ ID NO:3 and SEQ ID NO:4 or SEQ ID NO:5 and SEQ ID NO:6 or SEQ ID NO:7 and SEQ ID NO:8 or

30   SEQ ID NO:9 and SEQ ID NO:10.

The phrase "functional effects" in the context of assays for testing compounds affecting a channel comprising Kv6.2 includes the determination of any parameter that is indirectly or directly under the influence of the channel. It includes changes in ion flux and membrane potential, and also includes other physiologic effects

5   such as increases or decreases of transcription or hormone release.

"Determining the functional effect" refers to examining the effect of a compound that increases or decreases ion flux on a cell or cell membrane in terms of cell and cell membrane function. The ion flux can be any ion that passes through a channel and analogues thereof, e.g., potassium, rubidium, sodium. Preferably, the term refers to

10   the functional effect of the compound on the channels comprising Kv6.2, e.g., changes in ion flux including radioisotopes, current amplitude, membrane potential, current flow, transcription, protein binding, phosphorylation, dephosphorylation, second messenger concentrations (cAMP, cGMP, Ca^{2+}, IP_3) and other physiological effects such as hormone and neurotransmitter release, as well as changes in voltage and current. Such functional

15   effects can be measured by any means known to those skilled in the art, e.g., patch clamping, voltage-sensitive dyes, whole cell currents, radioisotope efflux, inducible markers, and the like.

"Inhibitors," "activators" or "modulators" of voltage-gated potassium channels comprising Kv6.2 refer to inhibitory or activating molecules identified using *in*

20   *vitro* and *in vivo* assays for Kv6.2 channel function. Inhibitors are compounds that decrease, block, prevent, delay activation, inactivate, desensitize, or down regulate the channel. Activators are compounds that increase, open, activate, facilitate, enhance activation, sensitize or up regulate channel activity. Such assays for inhibitors and activators include *e.g.*, expressing Kv6.2 in cells or cell membranes and then measuring

25   flux of ions through the channel and determining changes in polarization (i.e., electrical potential). Alternatively, cells expressing endogenous Kv6.2 channels can be used in such assays. To examine the extent of inhibition, samples or assays comprising an Kv6.2 channel are treated with a potential activator or inhibitor and are compared to control samples without the inhibitor. Control samples (untreated with inhibitors) are assigned a

30   relative Kv6.2 activity value of 100%. Inhibition of channels comprising Kv6.2 is achieved when the Kv6.2 activity value relative to the control is about 90%, preferably 50%, more preferably 25-0%. Activation of channels comprising Kv6.2 is achieved when

the Kv6.2 activity value relative to the control is 110%, more preferably 150%, most preferably at least 200-500% higher or 1000% or higher.

"Biologically active" Kv6.2 refers to Kv6.2 that has the ability to form a potassium channel having the characteristic of voltage-gating tested as described above.

5   The terms "isolated," "purified," or "biologically pure" refer to material that is substantially or essentially free from components that normally accompany it as found in its native state. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein that is the predominant species present in
10   a preparation is substantially purified. In particular, an isolated Kv6.2 nucleic acid is separated from open reading frames that flank the Kv6.2 gene and encode proteins other than Kv6.2. The term "purified" denotes that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. Particularly, it means that the nucleic acid or protein is at least 85% pure, more preferably at least 95% pure, and most preferably at
15   least 99% pure.

"Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which
20   have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs).

Unless otherwise indicated, a particular nucleic acid sequence also
25   implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991);
30   Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)). The term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

A particular nucleic acid sequence also implicitly encompasses "splice variants." Similarly, a particular protein encoded by a nucleic acid implicitly encompasses any protein encoded by a splice variant of that nucleic acid. "Splice variants," as the name suggests, are products of alternative splicing of a gene. After

5　transcription, an initial nucleic acid transcript may be spliced such that different (alternate) nucleic acid splice products encode different polypeptides. Mechanisms for the production of splice variants vary, but include alternate splicing of exons. Alternate polypeptides derived from the same nucleic acid by read-through transcription are also encompassed by this definition. Any products of a splicing reaction, including

10　recombinant forms of the splice products, are included in this definition. An example of potassium channel splice variants is discussed in Leicher, *et al.*, *J. Biol. Chem.* 273(52):35095-35101 (1998).

The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid

15　polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner

20　similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and

25　an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a

30　manner similar to a naturally occurring amino acid.

Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB

Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

"Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively

5   modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine.

10   Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill

15   will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

20   As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid.

25   Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

The following eight groups each contain amino acids that are conservative substitutions for one another:

30   1)   Alanine (A), Glycine (G);

       2)   Aspartic acid (D), Glutamic acid (E);

       3)   Asparagine (N), Glutamine (Q);

4) Arginine (R), Lysine (K);

5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);

6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);

7) Serine (S), Threonine (T); and

8) Cysteine (C), Methionine (M)

(see, e.g., Creighton, *Proteins* (1984)).

Macromolecular structures such as polypeptide structures can be described in terms of various levels of organization. For a general discussion of this organization, see, e.g., Alberts et al., *Molecular Biology of the Cell* (3rd ed., 1994) and Cantor and Schimmel, *Biophysical Chemistry Part I: The Conformation of Biological Macromolecules* (1980). "Primary structure" refers to the amino acid sequence of a particular peptide. "Secondary structure" refers to locally ordered, three dimensional structures within a polypeptide. These structures are commonly known as domains. Domains are portions of a polypeptide that form a compact unit of the polypeptide and are typically 50 to 350 amino acids long. Typical domains are made up of sections of lesser organization such as stretches of β-sheet and α-helices. "Tertiary structure" refers to the complete three dimensional structure of a polypeptide monomer. "Quaternary structure" refers to the three dimensional structure formed by the noncovalent association of independent tertiary units. Anisotropic terms are also known as energy terms.

A "label" is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include ^{32}P, fluorescent dyes, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin, digoxigenin, or haptens and proteins for which antisera or monoclonal antibodies are available (e.g., the polypeptide of SEQ ID NO:1 can be made detectable, e.g., by incorporating a radiolabel into the peptide, and used to detect antibodies specifically reactive with the peptide).

As used herein a "nucleic acid probe or oligonucleotide" is defined as a nucleic acid capable of binding to a target nucleic acid of complementary sequence through one or more types of chemical bonds, usually through complementary base pairing, usually through hydrogen bond formation. As used herein, a probe may include natural (i.e., A, G, C, or T) or modified bases (7-deazaguanosine, inosine, etc.). In

addition, the bases in a probe may be joined by a linkage other than a phosphodiester bond, so long as it does not interfere with hybridization. Thus, for example, probes may be peptide nucleic acids in which the constituent bases are joined by peptide bonds rather than phosphodiester linkages. It will be understood by one of skill in the art that probes

5   may bind target sequences lacking complete complementarity with the probe sequence depending upon the stringency of the hybridization conditions. The probes are preferably directly labeled as with isotopes, chromophores, lumiphores, chromogens, or indirectly labeled such as with biotin to which a streptavidin complex may later bind. By assaying for the presence or absence of the probe, one can detect the presence or absence of the

10   select sequence or subsequence.

A "labeled nucleic acid probe or oligonucleotide" is one that is bound, either covalently, through a linker or a chemical bond, or noncovalently, through ionic, van der Waals, electrostatic, or hydrogen bonds to a label such that the presence of the probe may be detected by detecting the presence of the label bound to the probe.

15   The term "recombinant" when used with reference, e.g., to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within

20   the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all.

A "promoter" is defined as an array of nucleic acid control sequences that direct transcription of a nucleic acid. As used herein, a promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a

25   polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. A "constitutive" promoter is a promoter that i. active under most environmental and developmental conditions. An "inducible" promoter is a promoter that is active under environmental or developmental regulation.

30   The term "operably linked" refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter, or array of transcription factor binding

sites) and a second nucleic acid sequence, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

The term "heterologous" when used with reference to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not

5   found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid, e.g., a promoter from one source and a coding region from another source. Similarly, a heterologous protein indicates that the protein comprises two or more subsequences that are not found in the same relationship to

10   each other in nature (e.g., a fusion protein).

An "expression vector" is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a host cell. The expression vector can be part of a plasmid, virus, or nucleic acid fragment. Typically, the expression vector

15   includes a nucleic acid to be transcribed operably linked to a promoter.

The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (i.e., 70% identity, preferably 75%, 80%, 85%, 90%, or 95% identity over a

20   specified region such as the Kv6.2 subunit association region or the S4-S6 region), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Such sequences are then said to be "substantially identical." This definition also refers to the compliment of a test

25   sequence. Preferably, the identity exists over a region that is at least about 25 amino acids or nucleotides in length, or more preferably over a region that is 50-100 amino acids or nucleotides in length.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison

30   algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be

designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

A "comparison window", as used herein, includes reference to a segment

5   of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of

10   sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and

15   TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (*see, e.g., Current Protocols in Molecular Biology* (Ausubel *et al.*, eds. 1995 supplement)).

One example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise

20   alignments to show relationship and percent sequence identity. It also plots a tree or dendogram showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle, *J. Mol. Evol.* 35:351-360 (1987). The method used is similar to the method described by Higgins & Sharp, *CABIOS* 5:151-153 (1989). The program can align up to 300 sequences, each

25   of a maximum length of 5,000 nucleotides or amino acids. The multiple alignment procedure begins with the pairwise alignment of the two most similar sequences, producing a cluster of two aligned sequences. This cluster is then aligned to the next most related sequence or cluster of aligned sequences. Two clusters of sequences are aligned by a simple extension of the pairwise alignment of two individual sequences. The

30   final alignment is achieved by a series of progressive, pairwise alignments. The program is run by designating specific sequences and their amino acid or nucleotide coordinates for regions of sequence comparison and by designating the program parameters. Using

PILEUP, a reference sequence is compared to other test sequences to determine the percent sequence identity relationship using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps. PILEUP can be obtained from the GCG sequence analysis software package, *e.g.*, version 7.0 (Devereaux *et al.,*

5   *Nuc. Acids Res.* 12:387-395 (1984).

Another example of algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al., Nuc. Acids Res.* 25:3389-3402 (1977) and Altschul *et al., J. Mol. Biol.* 215:403-410 (1990), respectively. Software for performing BLAST

10   analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood

15   word score threshold (Altschul *et al., supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N

20   (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either

25   sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) or 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix

30   (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by

5   which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

10   An indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ

15   only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules or their complements hybridize to each other under stringent conditions, as described below. Yet another indication that two nucleic acid sequences are substantially identical is that the same primers can be used to amplify the sequence.

20   The phrase "selectively (or specifically) hybridizes to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent hybridization conditions when that sequence is present in a complex mixture (e.g., total cellular or library DNA or RNA).

The phrase "stringent hybridization conditions" refers to conditions under

25   which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic acid, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, *Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic*

30   *Probes*, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10°C lower than the

thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are

5   occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the

10   addition of destabilizing agents such as formamide. For high stringency hybridization, a positive signal is at least two times background, preferably 10 times background hybridization. Exemplary high stringency or stringent hybridization conditions include: 50% formamide, 5x SSC and 1% SDS incubated at 42° C or 5x SSC and 1% SDS incubated at 65° C, with a wash in 0.2x SSC and 0.1% SDS at 65° C.

15   Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides that they encode are substantially identical. This occurs, for example, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. In such cased, the nucleic acids typically hybridize under moderately stringent hybridization conditions. Exemplary

20   "moderately stringent hybridization conditions" include a hybridization in a buffer of 40% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 1X SSC at 45°C. A positive hybridization is at least twice background. Those of ordinary skill will readily recognize that alternative hybridization and wash conditions can be utilized to provide conditions of similar stringency.

25   "Antibody" refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or

30   lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids

5   primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

Antibodies exist, e.g., as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to

10   produce F(ab)'$_2$, a dimer of Fab which itself is a light chain joined to V_H-C_H1 by a disulfide bond. The F(ab)'$_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the F(ab)'$_2$ dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region (*see Fundamental Immunology* (Paul ed., 3d ed. 1993). While various antibody fragments are defined in

15   terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized *de novo* using recombinant DNA methodologies (e.g., single chain Fv) or those identified using

20   phage display libraries (*see, e.g.*, McCafferty *et al.*, *Nature* 348:552-554 (1990))

For preparation of monoclonal or polyclonal antibodies, any technique known in the art can be used (*see, e.g.*, Kohler & Milstein, *Nature* 256:495-497 (1975); Kozbor *et al.*, *Immunology Today* 4: 72 (1983); Cole *et al.*, pp. 77-96 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. (1985)). Techniques for the

25   production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized antibodies. Alternatively, phage display technology can be used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens (*see, e.g.*, McCafferty *et al.*, *Nature*

30   348:552-554 (1990); Marks *et al.*, *Biotechnology* 10:779-783 (1992)).

An "anti- Kv6.2" antibody is an antibody or antibody fragment that specifically binds a polypeptide encoded by the Kv6.2 gene, cDNA, or a subsequence thereof.

A "chimeric antibody" is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity.

The term "immunoassay" is an assay that uses an antibody to specifically bind an antigen. The immunoassay is characterized by the use of specific binding properties of a particular antibody to isolate, target, and/or quantify the antigen.

The phrase "specifically (or selectively) binds" to an antibody or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and do not substantially bind in a significant amount to other proteins present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, polyclonal antibodies raised to Kv6.2, having the sequence of SEQ ID NO:1 or SEQ ID NO:17, encoded by SEQ NO:2 or SEQ ID NO:18, splice variants, or portions thereof, can be selected to obtain only those polyclonal antibodies that are specifically immunoreactive with Kv6.2 and not with other proteins, except for polymorphic variants, orthologs, and alleles of Kv6.2. This selection may be achieved by subtracting out antibodies that cross-react with molecules such as Kv6.1, other Kv6.2 orthologs, and with other Kv6 family members or other Kv superfamily members . A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (*see, e.g.*, Harlow & Lane, *Antibodies, A Laboratory Manual* (1988) for a description of immunoassay formats and

conditions that can be used to determine specific immunoreactivity). Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 to 100 times background.

5   The phrase "selectively associates with" refers to the ability of a nucleic acid to "selectively hybridize" with another as defined above, or the ability of an antibody to "selectively (or specifically) bind to a protein, as defined above.

By "host cell" is meant a cell that contains an expression vector and supports the replication or expression of the expression vector. Host cells may be prokaryotic cells such as *E. coli*, or eukaryotic cells such as yeast, insect, amphibian, or

10   mammalian cells such as CHO, HeLa and the like, e.g., cultured cells, explants, and cells *in vivo*.

"Biological sample" as used herein is a sample of biological tissue or fluid that contains Kv6.2 or nucleic acid encoding Kv6.2 protein. Such samples include, but are not limited to, tissue isolated from humans. Biological samples may also include

15   sections of tissues such as frozen sections taken for histologic purposes. A biological sample is typically obtained from a eukaryotic organism, preferably eukaryotes such as fungi, plants, insects, protozoa, birds, fish, reptiles, and preferably a mammal such as rat, mice, cow, dog, guinea pig, or rabbit, and most preferably a primate such as chimpanzees, or humans.

20

III.   ISOLATING THE GENE ENCODING Kv6.2

A.   General recombinant DNA methods

This invention relies on routine techniques in the field of recombinant genetics. Basic texts disclosing the general methods of use in this invention include

25   Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* (2nd ed. 1989); Kriegler, *Gene Transfer and Expression: A Laboratory Manual* (1990); and *Current Protocols in Molecular Biology* (Ausubel *et al.*, eds., 1994)).

For nucleic acids, sizes are given in either kilobases (kb) or base pairs (bp). These are estimates derived from agarose or acrylamide gel electrophoresis, from

30   sequenced nucleic acids, or from published DNA sequences. For proteins, sizes are given in kilodaltons (kDa) or amino acid residue numbers. Proteins sizes are estimated from gel

electrophoresis, from sequenced proteins, from derived amino acid sequences, or from
published protein sequences.

Oligonucleotides that are not commercially available can be chemically
synthesized according to the solid phase phosphoramidite triester method first described

5   by Beaucage & Caruthers, *Tetrahedron Letts*. 22:1859-1862 (1981), using an automated
synthesizer, as described in Van Devanter *et. al., Nucleic Acids Res.* 12:6159-6168
(1984). Purification of oligonucleotides is by either native acrylamide gel electrophoresis
or by anion-exchange HPLC as described in Pearson & Reanier, *J. Chrom*. 255:137-149
(1983).

10   The sequence of the cloned genes and synthetic oligonucleotides can be
verified after cloning using, e.g., the chain termination method for sequencing double-
stranded templates of Wallace *et al., Gene* 16:21-26 (1981).

B.   *Cloning methods for the isolation of nucleotide sequences encoding Kv6.2*

15   In general, the nucleic acid sequences encoding Kv6.2 and related nucleic
acid sequence homologs are cloned from cDNA and genomic DNA libraries or isolated
using amplification techniques with oligonucleotide primers. For example, Kv6.2
sequences are typically isolated from human nucleic acid (genomic or cDNA) libraries by
hybridizing with a nucleic acid probe or polynucleotide, the sequence of which can be

20   derived from SEQ ID NO:2 or SEQ ID NO:18, preferably from the region encoding the
subunit association region or the S4-S6 region. A suitable tissue from which Kv6.2
RNA and cDNA can be isolated is brain tissue such as whole brain.

Amplification techniques using primers can also be used to amplify and
isolate Kv6.2 from DNA or RNA. The following primers can also be used to amplify a

25   sequence of Kv6.2:
ATGCCCATGTCTTCCAGAGACAGG (SEQ ID NO:3),
GATGTCTAGAGGGAGTTACATGTAGCG (SEQ ID NO:4) and
GGCACTACGCATCCTCTACGTAATGCGC (SEQ ID NO:5),
GATGATGGCCCACCAATAGGATGCGG (SEQ ID NO:6) and

30   ATGCCCATGCCTTCCAGAGACGG (SEQ ID NO:7),
TTACATGTGCATGATAGGCAAGGCTG (SEQ ID NO:8) and

GTCCAGGCCCAAGACAAGTGTCAG (SEQ ID NO:9),

GGGAGAAGGTGTGGAAGATAGACG (SEQ ID NO:10).

These primers can be used, e.g., to amplify either the full length sequence or a probe of one to several hundred nucleotides, which is then used to screen a human library for full-length Kv6.2.

Nucleic acids encoding Kv6.2 can also be isolated from expression libraries using antibodies as probes. Such polyclonal or monoclonal antibodies can be raised using the sequence of SEQ ID NO:1, SEQ ID NO:17, or an immunogenic portion thereof.

Kv6.2 polymorphic variants, orthologs, and alleles that are substantially identical to the subunit association region or the S4-S6 region of Kv6.2 can be isolated using Kv6.2 nucleic acid probes and oligonucleotides under stringent hybridization conditions, by screening libraries. Alternatively, expression libraries can be used to clone Kv6.2 and Kv6.2 polymorphic variants, orthologs, and alleles by detecting expressed homologs immunologically with antisera or purified antibodies made against Kv6.2 or portions thereof (e.g., the subunit association region or the S4-S6 region of Kv6.2), which also recognize and selectively bind to the Kv6.2 homolog.

To make a cDNA library, one should choose a source that is rich in Kv6.2 mRNA, e.g., tissue such as whole brain. The mRNA is then made into cDNA using reverse transcriptase, ligated into a recombinant vector, and transfected into a recombinant host for propagation, screening and cloning. Methods for making and screening cDNA libraries are well known (see, e.g., Gubler & Hoffman, Gene 25:263-269 (1983); Sambrook et al., supra; Ausubel et al., supra).

For a genomic library, the DNA is extracted from the tissue and either mechanically sheared or enzymatically digested to yield fragments of about 12-20 kb. The fragments are then separated by gradient centrifugation from undesired sizes and are constructed in bacteriophage lambda vectors. These vectors and phage are packaged in vitro. Recombinant phage are analyzed by plaque hybridization as described in Benton & Davis, Science 196:180-182 (1977). Colony hybridization is carried out as generally described in Grunstein et al., Proc. Natl. Acad. Sci. USA, 72:3961-3965 (1975).

An alternative method of isolating Kv6.2 nucleic acid and its homologs combines the use of synthetic oligonucleotide primers and amplification of an RNA or DNA template (*see* U.S. Patents 4,683,195 and 4,683,202; *PCR Protocols: A Guide to Methods and Applications* (Innis *et al.*, eds, 1990)). Methods such as polymerase chain

5   reaction (PCR) and ligase chain reaction (LCR) can be used to amplify nucleic acid sequences of Kv6.2 directly from mRNA, from cDNA, from genomic libraries or cDNA libraries. Degenerate oligonucleotides can be designed to amplify Kv6.2 homologs using the sequences provided herein. Restriction endonuclease sites can be incorporated into the primers. Polymerase chain reaction or other *in vitro* amplification methods may also

10   be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of Kv6.2 encoding mRNA in physiological samples, for nucleic acid sequencing, or for other purposes. Genes amplified by the PCR reaction can be purified from agarose gels and cloned into an appropriate vector.

15   Gene expression of Kv6.2 can also be analyzed by techniques known in the art, e.g., reverse transcription and amplification of mRNA, isolation of total RNA or poly A^+ RNA, northern blotting, dot blotting, *in situ* hybridization, RNase protection, high density polynucleotide array technology and the like.

Synthetic oligonucleotides can be used to construct recombinant Kv6.2

20   genes for use as probes or for expression of protein. This method is performed using a series of overlapping oligonucleotides usually 40-120 bp in length, representing both the sense and nonsense strands of the gene. These DNA fragments are then annealed, ligated and cloned. Alternatively, amplification techniques can be used with precise primers to amplify a specific subsequence of the Kv6.2 gene. The specific subsequence is then

25   ligated into an expression vector.

The gene for Kv6.2 is typically cloned into intermediate vectors before transformation into prokaryotic or eukaryotic cells for replication and/or expression. These intermediate vectors are typically prokaryote vectors, e.g., plasmids, or shuttle vectors.

30

C.   *Expression in prokaryotes and eukaryotes*

To obtain high level expression of a cloned gene, such as those cDNAs encoding Kv6.2, one typically subclones Kv6.2 into an expression vector that contains a strong promoter to direct transcription, a transcription/translation terminator, and if for a

5   nucleic acid encoding a protein, a ribosome binding site for translational initiation. Suitable bacterial promoters are well known in the art and described, e.g., in Sambrook *et al.* and Ausubel *et al, supra*. Bacterial expression systems for expressing the Kv6.2 protein are available in, e.g., *E. coli, Bacillus sp.,* and *Salmonella* (Palva *et al., Gene* 22:229-235 (1983); Mosbach *et al., Nature* 302:543-545 (1983). Kits for such expression

10   systems are commercially available. Eukaryotic expression systems for mammalian cells, yeast, and insect cells are well known in the art and are also commercially available.

Selection of the promoter used to direct expression of a heterologous nucleic acid depends on the particular application. The promoter is preferably positioned about the same distance from the heterologous transcription start site as it is from the

15   transcription start site in its natural setting. As is known in the art, however, some variation in this distance can be accommodated without loss of promoter function.

In addition to the promoter, the expression vector typically contains a transcription unit or expression cassette that contains all the additional elements required for the expression of the Kv6.2 encoding nucleic acid in host cells. A typical expression

20   cassette thus contains a promoter operably linked to the nucleic acid sequence encoding Kv6.2 and signals required for efficient polyadenylation of the transcript, ribosome binding sites, and translation termination. Additional elements of the cassette may include enhancers and, if genomic DNA is used as the structural gene, introns with functional splice donor and acceptor sites.

25   In addition to a promoter sequence, the expression cassette should also contain a transcription termination region downstream of the structural gene to provide for efficient termination. The termination region may be obtained from the same gene as the promoter sequence or may be obtained from different genes.

The particular expression vector used to transport the genetic information

30   into the cell is not particularly critical. Any of the conventional vectors used for expression in eukaryotic or prokaryotic cells may be used. Standard bacterial expression vectors include plasmids such as pBR322 based plasmids, pSKF, pET23D, and fusion

expression systems such as GST and LacZ. Epitope tags can also be added to recombinant proteins to provide convenient methods of isolation, e.g., c-myc.

Expression vectors containing regulatory elements from eukaryotic viruses are typically used in eukaryotic expression vectors, e.g., SV40 vectors, papilloma virus

5   vectors, and vectors derived from Epstein-Barr virus. Other exemplary eukaryotic vectors include pMSG, pAV009/A$^+$, pMTO10/A$^+$, pMAMneo-5, baculovirus pDSVE, and any other vector allowing expression of proteins under the direction of the CMV promoter, SV40 early promoter, SV40 later promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or

10   other promoters shown effective for expression in eukaryotic cells.

Some expression systems have markers that provide gene amplification such as thymidine kinase and dihydrofolate reductase. Alternatively, high yield expression systems not involving gene amplification are also suitable, such as using a baculovirus vector in insect cells, with a Kv6.2 encoding sequence under the direction of

15   the polyhedrin promoter or other strong baculovirus promoters.

The elements that are typically included in expression vectors also include a replicon that functions in E. coli, a gene encoding antibiotic resistance to permit selection of bacteria that harbor recombinant plasmids, and unique restriction sites in nonessential regions of the plasmid to allow insertion of eukaryotic sequences. The

20   particular antibiotic resistance gene chosen is not critical, any of the many resistance genes known in the art are suitable. The prokaryotic sequences are preferably chosen such that they do not interfere with the replication of the DNA in eukaryotic cells, if necessary.

Standard transfection methods are used to produce bacterial, mammalian,

25   yeast or insect cell lines that express large quantities of Kv6.2 protein, which are then purified using standard techniques (see, e.g., Colley et al., J. Biol. Chem. 264:17619-17622 (1989); Guide to Protein Purification, in Methods in Enzymology, vol. 182 (Deutscher, ed., 1990)). Transformation of eukaryotic and prokaryotic cells are performed according to standard techniques (see, e.g., Morrison, J. Bact. 132:349-351

30   (1977); Clark-Curtiss & Curtiss, Methods in Enzymology 101:347-362 (Wu et al., eds, 1983).

Any of the well-known procedures for introducing foreign nucleotide sequences into host cells may be used. These include the use of calcium phosphate transfection, polybrene, protoplast fusion, electroporation, liposomes, microinjection, plasma vectors, viral vectors and any of the other well known methods for introducing

5   cloned genomic DNA, cDNA, synthetic DNA or other foreign genetic material into a host cell (*see, e.g.*, Sambrook *et al., supra*). It is only necessary that the particular genetic engineering procedure used be capable of successfully introducing at least one gene into the host cell capable of expressing Kv6.2.

After the expression vector is introduced into the cells, the transfected cells

10   are cultured under conditions favoring expression of Kv6.2, which is recovered from the culture using standard techniques identified below.

IV.   PURIFICATION OF Kv6.2 POLYPEPTIDES

Either naturally occurring or recombinant Kv6.2 can be purified for use in

15   functional assays. Naturally occurring Kv6.2 monomers can be purified, e.g., from human tissue such as whole brain, and any other source of a Kv6.2 homolog. Recombinant Kv6.2 monomers can be purified from any suitable expression system.

The Kv6.2 monomers may be purified to substantial purity by standard techniques, including selective precipitation with such substances as ammonium sulfate;

20   column chromatography, immunopurification methods, and others (*see, e.g.*, Scopes, *Protein Purification: Principles and Practice* (1982); U.S. Patent No. 4,673,641; Ausubel *et al., supra;* and Sambrook *et al., supra*).

A number of procedures can be employed when recombinant Kv6.2 monomers are being purified. For example, proteins having established molecular

25   adhesion properties can be reversible fused to the Kv6.2 monomers. With the appropriate ligand, the Kv6.2 monomers can be selectively adsorbed to a purification column and then freed from the column in a relatively pure form. The fused protein is then removed by enzymatic activity. Finally the Kv6.2 monomers could be purified using immunoaffinity columns.

30

A.   *Purification of Kv6.2 monomers from recombinant bacteria*

Recombinant proteins are expressed by transformed bacteria in large amounts, typically after promoter induction; but expression can be constitutive. Promoter induction with IPTG is a one example of an inducible promoter system. Bacteria are

5   grown according to standard procedures in the art. Fresh or frozen bacteria cells are used for isolation of protein.

Proteins expressed in bacteria may form insoluble aggregates ("inclusion bodies"). Several protocols are suitable for purification of the Kv6.2 monomers inclusion bodies. For example, purification of inclusion bodies typically involves the extraction,

10   separation and/or purification of inclusion bodies by disruption of bacterial cells, e.g., by incubation in a buffer of 50 mM TRIS/HCL pH 7.5, 50 mM NaCl, 5 mM MgCl$_2$, 1 mM DTT, 0.1 mM ATP, and 1 mM PMSF. The cell suspension can be lysed using 2-3 passages through a French Press, homogenized using a Polytron (Brinkman Instruments) or sonicated on ice. Alternate methods of lysing bacteria are apparent to those of skill in

15   the art (*see, e.g.,* Sambrook *et al., supra*; Ausubel *et al., supra*).

If necessary, the inclusion bodies are solubilized, and the lysed cell suspension is typically centrifuged to remove unwanted insoluble matter. Proteins that formed the inclusion bodies may be renatured by dilution or dialysis with a compatible buffer. Suitable solvents include, but are not limited to urea (from about 4 M to about 8

20   M), formamide (at least about 80%, volume/volume basis), and guanidine hydrochloride (from about 4 M to about 8 M). Some solvents which are capable of solubilizing aggregate-forming proteins, for example SDS (sodium dodecyl sulfate), 70% formic acid, are inappropriate for use in this procedure due to the possibility of irreversible denaturation of the proteins, accompanied by a lack of immunogenicity and/or activity.

25   Although guanidine hydrochloride and similar agents are denaturants, this denaturation is not irreversible and renaturation may occur upon removal (by dialysis, for example) or dilution of the denaturant, allowing re-formation of immunologically and/or biologically active protein. Other suitable buffers are known to those skilled in the art. Human Kv6.2 monomers are separated from other bacterial proteins by standard separation techniques,

30   e.g., with Ni-NTA agarose resin.

Alternatively, it is possible to purify the Kv6.2 monomers from bacteria periplasm. After lysis of the bacteria, when the Kv6.2 monomers are exported into the

periplasm of the bacteria, the periplasmic fraction of the bacteria can be isolated by cold osmotic shock in addition to other methods known to skill in the art. To isolate recombinant proteins from the periplasm, the bacterial cells are centrifuged to form a pellet. The pellet is resuspended in a buffer containing 20% sucrose. To lyse the cells, the bacteria are centrifuged and the pellet is resuspended in ice-cold 5 mM $MgSO_4$ and kept in an ice bath for approximately 10 minutes. The cell suspension is centrifuged and the supernatant decanted and saved. The recombinant proteins present in the supernatant can be separated from the host proteins by standard separation techniques well known to those of skill in the art.

B.   *Standard protein separation techniques for purifying the Kv6.2 monomers*

Solubility fractionation

Often as an initial step, particularly if the protein mixture is complex, an initial salt fractionation can separate many of the unwanted host cell proteins (or proteins derived from the cell culture media) from the recombinant protein of interest. The preferred salt is ammonium sulfate. Ammonium sulfate precipitates proteins by effectively reducing the amount of water in the protein mixture. Proteins then precipitate on the basis of their solubility. The more hydrophobic a protein is, the more likely it is to precipitate at lower ammonium sulfate concentrations. A typical protocol includes adding saturated ammonium sulfate to a protein solution so that the resultant ammonium sulfate concentration is between 20-30%. This concentration will precipitate the most hydrophobic of proteins. The precipitate is then discarded (unless the protein of interest is hydrophobic) and ammonium sulfate is added to the supernatant to a concentration known to precipitate the protein of interest. The precipitate is then solubilized in buffer and the excess salt removed if necessary, either through dialysis or diafiltration. Other methods that rely on solubility of proteins, such as cold ethanol precipitation, are well known to those of skill in the art and can be used to fractionate complex protein mixtures.

Size differential filtration

The molecular weight of the Kv6.2 monomers can be used to isolated it from proteins of greater and lesser size using ultrafiltration through membranes of different pore size (for example, Amicon or Millipore membranes). As a first step, the

protein mixture is ultrafiltered through a membrane with a pore size that has a lower molecular weight cut-off than the molecular weight of the protein of interest. The retentate of the ultrafiltration is then ultrafiltered against a membrane with a molecular cut off greater than the molecular weight of the protein of interest. The recombinant

5   protein will pass through the membrane into the filtrate. The filtrate can then be chromatographed as described below.

Column chromatography

The Kv6.2 monomers can also be separated from other proteins on the

10   basis of its size, net surface charge, hydrophobicity, and affinity for ligands. In addition, antibodies raised against proteins can be conjugated to column matrices and the proteins immunopurified. All of these methods are well known in the art. It will be apparent to one of skill that chromatographic techniques can be performed at any scale and using equipment from many different manufacturers (e.g., Pharmacia Biotech).

15

V.   IMMUNOLOGICAL DETECTION OF Kv6.2

In addition to the detection of Kv6.2 genes and gene expression using nucleic acid hybridization technology, one can also use immunoassays to detect the Kv6.2 monomers. Immunoassays can be used to qualitatively or quantitatively analyze the

20   Kv6.2 monomers. A general overview of the applicable technology can be found in Harlow & Lane, *Antibodies: A Laboratory Manual* (1988).

A.   *Antibodies to Kv6.2 monomers*

Methods of producing polyclonal and monoclonal antibodies that react

25   specifically with the Kv6.2 monomers are known to those of skill in the art (*see, e.g.*, Coligan, *Current Protocols in Immunology* (1991); Harlow & Lane, *supra*; Goding, *Monoclonal Antibodies: Principles and Practice* (2d ed. 1986); and Kohler & Milstein, *Nature* 256:495-497 (1975). Such techniques include antibody preparation by selection of antibodies from libraries of recombinant antibodies in phage or similar vectors, as well

30   as preparation of polyclonal and monoclonal antibodies by immunizing rabbits or mice (*see, e.g.*, Huse *et al.*, *Science* 246:1275-1281 (1989); Ward *et al.*, *Nature* 341:544-546 (1989)).

A number of immunogens comprising portions of Kv6.2 monomers may be used to produce antibodies specifically reactive with Kv6.2 monomers. For example, recombinant Kv6.2 monomers or an antigenic fragment thereof, such as the subunit association region or the S4-S6 region, can be isolated as described herein. Recombinant

5   protein can be expressed in eukaryotic or prokaryotic cells as described above, and purified as generally described above. Recombinant protein is the preferred immunogen for the production of monoclonal or polyclonal antibodies. Alternatively, a synthetic peptide derived from the sequences disclosed herein and conjugated to a carrier protein can be used an immunogen. Naturally occurring protein may also be used either in pure

10   or impure form. The product is then injected into an animal capable of producing antibodies. Either monoclonal or polyclonal antibodies may be generated, for subsequent use in immunoassays to measure the protein.

Methods of production of polyclonal antibodies are known to those of skill in the art. An inbred strain of mice (e.g., BALB/C mice) or rabbits is immunized with the

15   protein using a standard adjuvant, such as Freund's adjuvant, and a standard immunization protocol. The animal's immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to the beta subunits. When appropriately high titers of antibody to the immunogen are obtained, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera

20   to enrich for antibodies reactive to the protein can be done if desired (*see* Harlow & Lane, *supra*).

Monoclonal antibodies may be obtained by various techniques familiar to those skilled in the art. Briefly, spleen cells from an animal immunized with a desired antigen are immortalized, commonly by fusion with a myeloma cell (*see* Kohler &

25   Milstein, *Eur. J. Immunol.* 6:511-519 (1976)). Alternative methods of immortalization include transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods well known in the art. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells may be enhanced

30   by various techniques, including injection into the peritoneal cavity of a vertebrate host. Alternatively, one may isolate DNA sequences which encode a monoclonal antibody or a

binding fragment thereof by screening a DNA library from human B cells according to the general protocol outlined by Huse *et al.*, *Science* 246:1275-1281 (1989).

Monoclonal antibodies and polyclonal sera are collected and titered against the immunogen protein in an immunoassay, for example, a solid phase immunoassay with the immunogen immobilized on a solid support. Typically, polyclonal antisera with a titer of 10^4 or greater are selected and tested for their cross reactivity against non-Kv proteins or other Kv6 family members such as Kv6.1 or other Kv superfamily members, using a competitive binding immunoassay. Specific polyclonal antisera and monoclonal antibodies will usually bind with a K_d of at least about 0.1 mM, more usually at least about 1 µM, preferably at least about 0.1 µM or better, and most preferably, 0.01 µM or better.

Once the specific antibodies against a Kv6.2 are available, the Kv6.2 can be detected by a variety of immunoassay methods. For a review of immunological and immunoassay procedures, see *Basic and Clinical Immunology* (Stites & Terr eds., 7th ed. 1991). Moreover, the immunoassays of the present invention can be performed in any of several configurations, which are reviewed extensively in Enzyme Immunoassay (Maggio, ed., 1980); and Harlow & Lane, *supra*.

B.   *Immunological binding assays*

The Kv6.2 can be detected and/or quantified using any of a number of well recognized immunological binding assays (*see, e.g.*, U.S. Patents 4,366,241; 4,376,110; 4,517,288; and 4,837,168). For a review of the general immunoassays, see also *Methods in Cell Biology: Antibodies in Cell Biology*, volume 37 (Asai, ed. 1993); *Basic and Clinical Immunology* (Stites & Terr, eds., 7th ed. 1991). Immunological binding assays (or immunoassays) typically use an antibody that specifically binds to a protein or antigen of choice (in this case the Kv6.2 or an antigenic subsequence thereof). The antibody (e.g., anti-Kv6.2) may be produced by any of a number of means well known to those of skill in the art and as described above.

Immunoassays also often use a labeling agent to specifically bind to and label the complex formed by the antibody and antigen. The labeling agent may itself be one of the moieties comprising the antibody/antigen complex. Thus, the labeling agent may be a labeled Kv6.2 polypeptide or a labeled anti-Kv6.2 antibody. Alternatively, the

labeling agent may be a third moiety, such a secondary antibody, which specifically binds to the antibody/Kv6.2 complex (a secondary antibody is typically specific to antibodies of the species from which the first antibody is derived). Other proteins capable of specifically binding immunoglobulin constant regions, such as protein A or protein G

5   may also be used as the label agent. These proteins exhibit a strong non-immunogenic reactivity with immunoglobulin constant regions from a variety of species (see, e.g., Kronval et al., J. Immunol. 111:1401-1406 (1973); Akerstrom et al., J. Immunol. 135:2589-2542 (1985)). The labeling agent can be modified with a detectable moiety, such as biotin, to which another molecule can specifically bind, such as streptavidin. A

10   variety of detectable moieties are well known to those skilled in the art.

Throughout the assays, incubation and/or washing steps may be required after each combination of reagents. Incubation steps can vary from about 5 seconds to several hours, preferably from about 5 minutes to about 24 hours. However, the incubation time will depend upon the assay format, antigen, volume of solution,

15   concentrations, and the like. Usually, the assays will be carried out at ambient temperature, although they can be conducted over a range of temperatures, such as 10°C to 40°C.

Non-competitive assay formats

20   Immunoassays for detecting the Kv6.2 in samples may be either competitive or noncompetitive. Noncompetitive immunoassays are assays in which the amount of antigen is directly measured. In one preferred "sandwich" assay, for example, the anti-Kv6.2 subunit antibodies can be bound directly to a solid substrate on which they are immobilized. These immobilized antibodies then capture Kv6.2 present in the test

25   sample. The Kv6.2 monomers are thus immobilized and then bound by a labeling agent, such as a second Kv6.2 antibody bearing a label. Alternatively, the second antibody may lack a label, but it may, in turn, be bound by a labeled third antibody specific to antibodies of the species from which the second antibody is derived. The second or third antibody is typically modified with a detectable moiety, such as biotin, to which another

30   molecule specifically binds, e.g., streptavidin, to provide a detectable moiety.

Competitive assay formats

In competitive assays, the amount of the Kv6.2 present in the sample is measured indirectly by measuring the amount of known, added (exogenous) Kv6.2 displaced (competed away) from an anti- Kv6.2 antibody by the unknown Kv6.2 present in a sample. In one competitive assay, a known amount of the Kv6.2 is added to a sample and the sample is then contacted with an antibody that specifically binds to the Kv6.2. The amount of exogenous Kv6.2 bound to the antibody is inversely proportional to the concentration of the Kv6.2 present in the sample. In a particularly preferred embodiment, the antibody is immobilized on a solid substrate. The amount of Kv6.2 bound to the antibody may be determined either by measuring the amount of Kv6.2 present in a Kv6.2/antibody complex, or alternatively by measuring the amount of remaining uncomplexed protein. The amount of Kv6.2 may be detected by providing a labeled Kv6.2 molecule.

A hapten inhibition assay is another preferred competitive assay. In this assay the known Kv6.2 is immobilized on a solid substrate. A known amount of anti-Kv6.2 antibody is added to the sample, and the sample is then contacted with the immobilized Kv6.2. The amount of anti- Kv6.2 antibody bound to the known immobilized Kv6.2 is inversely proportional to the amount of Kv6.2 present in the sample. Again, the amount of immobilized antibody may be detected by detecting either the immobilized fraction of antibody or the fraction of the antibody that remains in solution. Detection may be direct where the antibody is labeled or indirect by the subsequent addition of a labeled moiety that specifically binds to the antibody as described above.

Cross-reactivity determinations

Immunoassays in the competitive binding format can also be used for crossreactivity determinations for Kv6.2. For example, a protein have at least a partial sequence of SEQ ID NO:1 or SEQ ID NO:17, or a protein at least partially encoded by SEQ ID NO:2 or SEQ ID NO:18 or an immunogenic region thereof, such as the subunit association region or the S4-S6 region, can be immobilized to a solid support. Other proteins such as other Kv6 family members, e.g., Kv6.1 or Kv 6.2 orthologs, are added to the assay so as to compete for binding of the antisera to the immobilized antigen. The

ability of the added proteins to compete for binding of the antisera to the immobilized protein is compared to the ability of the Kv6.2 having a sequence of SEQ ID NO:1 or SEQ ID NO:17 to compete with itself. The percent crossreactivity for the above proteins is calculated, using standard calculations. Those antisera with less than 10%

5   crossreactivity with each of the added proteins listed above are selected and pooled. The cross-reacting antibodies are optionally removed from the pooled antisera by immunoabsorption with the added considered proteins, e.g., distantly related homologs.

The immunoabsorbed and pooled antisera are then used in a competitive binding immunoassay as described above to compare a second protein, thought to be

10   perhaps an allele, ortholog, or polymorphic variant of Kv6.2, to the immunogen protein. In order to make this comparison, the two proteins are each assayed at a wide range of concentrations and the amount of each protein required to inhibit 50% of the binding of the antisera to the immobilized protein is determined. If the amount of the second protein required to inhibit 50% of binding is less than 10 times the amount of the protein encoded

15   by Kv6.2 that is required to inhibit 50% of binding, then the second protein is said to specifically bind to the polyclonal antibodies generated to the respective Kv6.2 immunogen.

Other assay formats

20   Western blot (immunoblot) analysis is used to detect and quantify the presence of the Kv6.2 in the sample. The technique generally comprises separating sample proteins by gel electrophoresis on the basis of molecular weight, transferring the separated proteins to a suitable solid support, (such as a nitrocellulose filter, a nylon filter, or derivatized nylon filter), and incubating the sample with the antibodies that specifically

25   bind Kv6.2. The anti-Kv6.2 antibodies specifically bind to Kv6.2 on the solid support. These antibodies may be directly labeled or alternatively may be subsequently detected using labeled antibodies (e.g., labeled sheep anti-mouse antibodies) that specifically bind to the anti-Kv6.2 antibodies.

Other assay formats include liposome immunoassays (LIA), which use

30   liposomes designed to bind specific molecules (e.g., antibodies) and release encapsulated reagents or markers. The released chemicals are then detected according to standard techniques (*see* Monroe *et al.*, *Amer. Clin. Prod. Rev.* 5:34-41 (1986)).

Reduction of non-specific binding

One of skill in the art will appreciate that it is often desirable to minimize non-specific binding in immunoassays. Particularly, where the assay involves an antigen or antibody immobilized on a solid substrate it is desirable to minimize the amount of non-specific binding to the substrate. Means of reducing such non-specific binding are well known to those of skill in the art. Typically, this technique involves coating the substrate with a proteinaceous composition. In particular, protein compositions such as bovine serum albumin (BSA), nonfat powdered milk, and gelatin are widely used with powdered milk being most preferred.

Labels

The particular label or detectable group used in the assay is not a critical aspect of the invention, as long as it does not significantly interfere with the specific binding of the antibody used in the assay. The detectable group can be any material having a detectable physical or chemical property. Such detectable labels have been well-developed in the field of immunoassays and, in general, most any label useful in such methods can be applied to the present invention. Thus, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include magnetic beads (e.g., DYNABEADS^{TM}), fluorescent dyes (e.g., fluorescein isothiocyanate, Texas red, rhodamine, and the like), radiolabels (e.g., ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P), enzymes (e.g., horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic beads (e.g., polystyrene, polypropylene, latex, etc.).

The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. As indicated above, a wide variety of labels may be used, with the choice of label depending on sensitivity required, ease of conjugation with the compound, stability requirements, available instrumentation, and disposal provisions.

Non-radioactive labels are often attached by indirect means. Generally, a ligand molecule (e.g., biotin) is covalently bound to the molecule. The ligand then binds

to another molecules (e.g., streptavidin) molecule, which is either inherently detectable or covalently bound to a signal system, such as a detectable enzyme, a fluorescent compound, or a chemiluminescent compound. The ligands and their targets can be used in any suitable combination with antibodies that recognize Kv6.2, or secondary antibodies

5   that recognize anti-Kv6.2 antibodies.

The molecules can also be conjugated directly to signal generating compounds, e.g., by conjugation with an enzyme or fluorophore. Enzymes of interest as labels will primarily be hydrolases, particularly phosphatases, esterases and glycosidases, or oxidotases, particularly peroxidases. Fluorescent compounds include fluorescein and

10   its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, etc. Chemiluminescent compounds include luciferin, and 2,3-dihydrophthalazinediones, e.g., luminol. For a review of various labeling or signal producing systems that may be used, see U.S. Patent No. 4,391,904.

Means of detecting labels are well known to those of skill in the art. Thus,

15   for example, where the label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography. Where the label is a fluorescent label, it may be detected by exciting the fluorochrome with the appropriate wavelength of light and detecting the resulting fluorescence. The fluorescence may be detected visually, by means of photographic film, by the use of electronic detectors such

20   as charge coupled devices (CCDs) or photomultipliers and the like. Similarly, enzymatic labels may be detected by providing the appropriate substrates for the enzyme and detecting the resulting reaction product. Finally simple colorimetric labels may be detected simply by observing the color associated with the label. Thus, in various dipstick assays, conjugated gold often appears pink, while various conjugated beads

25   appear the color of the bead.

Some assay formats do not require the use of labeled components. For instance, agglutination assays can be used to detect the presence of the target antibodies. In this case, antigen-coated particles are agglutinated by samples comprising the target antibodies. In this format, none of the components need be labeled and the presence of

30   the target antibody is detected by simple visual inspection.

VI. ASSAYS FOR MODULATORS OF Kv6.2

A. Assays

Kv6.2 monomers and Kv6.2 alleles, orthologs, and polymorphic variants are subunits of voltaleg-gated potassium channels. The activity of a potassium channel comprising Kv6.2 can be assessed using a variety of *in vitro* and *in vivo* assays, e.g., measuring current, measuring membrane potential, measuring ion flux, e.g., potassium or rubidium, measuring potassium concentration, measuring second messengers and transcription levels, using potassium-dependent yeast growth assays, and using e.g., voltage-sensitive dyes, radioactive tracers, and patch-clamp electrophysiology.

Furthermore, such assays can be used to test for inhibitors and activators of channels comprising Kv6.2. Such modulators of a potassium channel are useful for treating various disorders involving potassium channels. Treatment of dysfunctions include, e.g., endocrine disorders, CNS disorders such as migraines, hearing and vision problems, psychotic disorders, seizures, and use as neuroprotective agents (e.g., to prevent stroke). Such modulators are also useful for investigation of the channel diversity provided by Kv6.2 and the regulation/modulation of potassium channel activity provided by Kv6.2.

Modulators of the potassium channels are tested using biologically active Kv6.2, either recombinant or naturally occurring. Kv6.2 can be isolated, co-expressed in a cell, or co-expressed in a membrane derived from a cell. In such assays, Kv6.2 is expressed alone to form a homomeric potassium channel or is preferably co-expressed with a second alpha subunit (e.g., another Kv superfamily member such as Kv2.1 or Kv2.2 or a Kv6 family member) so as to form a heteromeric potassium channel. Kv6.2 can also be expressed with additional beta subunits. Modulation is tested using one of the *in vitro* or *in vivo* assays described above. Samples or assays that are treated with a potential potassium channel inhibitor or activator are compared to control samples without the test compound, to examine the extent of modulation. Control samples (untreated with activators or inhibitors) are assigned a relative potassium channel activity value of 100. Inhibition of channels comprising Kv6.2 is achieved when the potassium channel activity value relative to the control is about 90%, preferably 50%, more preferably 25%. Activation of channels comprising Kv6.2 is achieved when the potassium channel activity value relative to the control is 110%, more preferably 150%,

more preferable 200% higher. Compounds that increase the flux of ions will cause a detectable increase in the ion current density by increasing the probability of a channel comprising Kv6.2 being open, by decreasing the probability of it being closed, by increasing conductance through the channel, and/or by allowing the passage of ions.

5   Changes in ion flux may be assessed by determining changes in polarization (i.e., electrical potential) of the cell or membrane expressing the potassium channel comprising Kv6.2. A preferred means to determine changes in cellular polarization is by measuring changes in current (thereby measuring changes in polarization) with voltage-clamp and patch-clamp techniques, e.g., the "cell-attached"

10   mode, the "inside-out" mode, and the "whole cell" mode (see, e.g., Ackerman et al., New Engl. J. Med. 336:1575-1595 (1997)). Whole cell currents are conveniently determined using the standard methodology (see, e.g., Hamil et al., PFlugers. Archiv. 391:85 (1981). Other known assays include: radiolabeled rubidium flux assays and fluorescence assays using voltage-sensitive dyes (see, e.g., Vestergarrd-Bogind et al., J. Membrane Biol.

15   88:67-75 (1988); Daniel et al., J. Pharmacol. Meth. 25:185-193 (1991); Holevinsky et al., J. Membrane Biology 137:59-70 (1994)). Assays for compounds capable of inhibiting or increasing potassium flux through the channel proteins comprising Kv6.2 can be performed by application of the compounds to a bath solution in contact with and comprising cells having a channel of the present invention (see, e.g., Blatz et al., Nature

20   323:718-720 (1986); Park, J. Physiol. 481:555-570 (1994)). Generally, the compounds to be tested are present in the range from 1 pM to 100 mM.

The effects of the test compounds upon the function of the channels can be measured by changes in the electrical currents or ionic flux or by the consequences of changes in currents and flux. Changes in electrical current or ionic flux are measured by

25   either increases or decreases in flux of ions such as potassium or rubidium ions. The cations can be measured in a variety of standard ways. They can be measured directly by concentration changes of the ions or indirectly by membrane potential or by radio-labeling of the ions. Consequences of the test compound on ion flux can be quite varied. Accordingly, any suitable physiological change can be used to assess the influence of a

30   test compound on the channels of this invention. The effects of a test compound can be measured by a toxin binding assay. When the functional consequences are determined using intact cells or animals, one can also measure a variety of effects such as transmitter

release (e.g., dopamine), hormone release (e.g., insulin), transcriptional changes to both known and uncharacterized genetic markers (e.g., northern blots), cell volume changes (e.g., in red blood cells), immunoresponses (e.g., T cell activation), changes in cell metabolism such as cell growth or pH changes, and changes in intracellular second

5   messengers such as Ca^{2+}, or cyclic nucleotides.

Preferably, the Kv6.2 that is a part of the potassium channel used in the assay will have the sequence displayed in SEQ ID NO:1, SEQ ID NO:17, or a conservatively modified variant thereof. Alternatively, the Kv6.2 of the assay will be derived from a eukaryote and include an amino acid subsequence having substantial

10   amino acid sequence identity to the subunit association region or the S4-S6 region of Kv6.2. Generally, the amino acid sequence identity will be at least 70%, preferably at least 75, 80, 85, 90%, most preferably at least 95%.

Kv6.2 orthologs will generally confer substantially similar properties on a channel comprising such Kv6.2, as described above. In a preferred embodiment, the cell

15   placed in contact with a compound that is suspected to be a Kv6.2 homolog is assayed for increasing or decreasing ion flux in a eukaryotic cell, e.g., an oocyte of *Xenopus* (e.g., *Xenopus laevis*) or a mammalian cell such as a CHO or HeLa cell. Channels that are affected by compounds in ways similar to Kv6.2 are considered homologs or orthologs of Kv6.2.

20

B.   *Modulators*

The compounds tested as modulators of Kv6.2 channels comprising a Kv6.2 subunit can be any small chemical compound, or a biological entity, such as a protein, sugar, nucleic acid or lipid. Alternatively, modulators can be genetically altered

25   versions of a human Kv6.2 subunit. Typically, test compounds will be small chemical molecules and peptides. Essentially any chemical compound can be used as a potential modulator or ligand in the assays of the invention, although most often compounds can be dissolved in aqueous or organic (especially DMSO-based) solutions are used. The assays are designed to screen large chemical libraries by automating the assay steps and

30   providing compounds from any convenient source to assays, which are typically run in parallel (e.g., in microtiter formats on microtiter plates in robotic assays). It will be appreciated that there are many suppliers of chemical compounds, including Sigma (St.

Louis, MO), Aldrich (St. Louis, MO), Sigma-Aldrich (St. Louis, MO), Fluka Chemika-Biochemica Analytika (Buchs Switzerland) and the like.

In one preferred embodiment, high throughput screening methods involve providing a combinatorial chemical or peptide library containing a large number of potential therapeutic compounds (potential modulator or ligand compounds). Such "combinatorial chemical libraries" or "ligand libraries" are then screened in one or more assays, as described herein, to identify those library members (particular chemical species or subclasses) that display a desired characteristic activity. The compounds thus identified can serve as conventional "lead compounds" or can themselves be used as potential or actual therapeutics.

A combinatorial chemical library is a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis, by combining a number of chemical "building blocks" such as reagents. For example, a linear combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks (amino acids) in every possible way for a given compound length (*i.e.*, the number of amino acids in a polypeptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks.

Preparation and screening of combinatorial chemical libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (*see, e.g.*, U.S. Patent 5,010,175, Furka, *Int. J. Pept. Prot. Res.* 37:487-493 (1991) and Houghton *et al.*, *Nature* 354:84-88 (1991)). Other chemistries for generating chemical diversity libraries can also be used. Such chemistries include, but are not limited to: peptoids (*e.g.*, PCT Publication No. WO 91/19735), encoded peptides (*e.g.*, PCT Publication No. WO 93/20242), random bio-oligomers (*e.g.*, PCT Publication No. WO 92/00091), benzodiazepines (*e.g.*, U.S. Pat. No. 5,288,514), diversomers such as hydantoins, benzodiazepines and dipeptides (Hobbs *et al.*, *Proc. Nat. Acad. Sci. USA* 90:6909-6913 (1993)), vinylogous polypeptides (Hagihara *et al.*, *J. Amer. Chem. Soc.* 114:6568 (1992)), nonpeptidal peptidomimetics with glucose scaffolding (Hirschmann *et al.*, *J. Amer. Chem. Soc.* 114:9217-9218 (1992)), analogous organic syntheses of small compound libraries (Chen *et al.*, *J. Amer. Chem. Soc.* 116:2661 (1994)), oligocarbamates (Cho *et al.*, *Science* 261:1303 (1993)), and/or peptidyl

phosphonates (Campbell *et al.*, *J. Org. Chem.* 59:658 (1994)), nucleic acid libraries (*see* Ausubel, Berger and Sambrook, all *supra*), peptide nucleic acid libraries (*see, e.g.*, U.S. Patent 5,539,083), antibody libraries (*see, e.g.*, Vaughn *et al.*, *Nature Biotechnology*, 14(3):309-314 (1996) and PCT/US96/10287), carbohydrate libraries (*see, e.g.*, Liang *et*

5   *al.*, *Science*, 274:1520-1522 (1996) and U.S. Patent 5,593,853), small organic molecule libraries (*see, e.g.*, benzodiazepines, Baum C&EN, Jan 18, page 33 (1993); isoprenoids, U.S. Patent 5,569,588; thiazolidinones and metathiazanones, U.S. Patent 5,549,974; pyrrolidines, U.S. Patents 5,525,735 and 5,519,134; morpholino compounds, U.S. Patent 5,506,337; benzodiazepines, 5,288,514, and the like).

10   Devices for the preparation of combinatorial libraries are commercially available (*see, e.g.*, 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY, Symphony, Rainin, Woburn, MA, 433A Applied Biosystems, Foster City, CA, 9050 Plus, Millipore, Bedford, MA). In addition, numerous combinatorial libraries are themselves commercially available (*see, e.g.*, ComGenex, Princeton, N.J., Asinex, Moscow, Ru,

15   Tripos, Inc., St. Louis, MO, ChemStar, Ltd, Moscow, RU, 3D Pharmaceuticals, Exton, PA, Martek Biosciences, Columbia, MD, etc.).

           In one embodiment, the invention provides solid phase based *in vitro* assays in a high throughput format, where the cell or tissue expressing a Kv6.2 channel comprising a human Kv6.2 subunit is attached to a solid phase substrate. In the high

20   throughput assays of the invention, it is possible to screen up to several thousand different modulators or ligands in a single day. In particular, each well of a microtiter plate can be used to run a separate assay against a selected potential modulator, or, if concentration or incubation time effects are to be observed, every 5-10 wells can test a single modulator. Thus, a single standard microtiter plate can assay about 96 modulators. If 1536 well

25   plates are used, then a single plate can easily assay from about 100- about 1500 different compounds. It is possible to assay several different plates per day; assay screens for up to about 6,000-20,000 different compounds is possible using the integrated systems of the invention.

30   **VII.   COMPUTER ASSISTED DRUG DESIGN USING Kv6.2**

           Yet another assay for compounds that modulate the activities of Kv6.2 involves computer assisted drug design, in which a computer system is used to generate a

three-dimensional structure of Kv6.2 based on the structural information encoded by the amino acid sequence. The input amino acid sequence interacts directly and actively with a pre-established algorithm in a computer program to yield secondary, tertiary, and quaternary structural models of the protein. The models of the protein structure are then

5 examined to identify regions of the structure that have the ability to bind, e.g., ligands or other potassium channel subunits. These regions are then used to identify ligands that bind to the protein or region where Kv6.2 interacts with other potassium channel subunits.

The three-dimensional structural model of the protein is generated by

10 entering channel protein amino acid sequences of at least 25, 50, 75 or 100 amino acid residues or corresponding nucleic acid sequences encoding an Kv6.2 monomer into the computer system. The amino acid sequence of each of the monomers is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:17, and a conservatively modified versions thereof. The amino acid sequence represents the primary sequence or

15 subsequence of each of the proteins, which encodes the structural information of the protein. At least 25, 50, 75, or 100 residues of the amino acid sequence (or a nucleotide sequence encoding at least about 25, 50, 75 or 100 amino acids) are entered into the computer system from computer keyboards, computer readable substrates that include, but are not limited to, electronic storage media (e.g., magnetic diskettes, tapes, cartridges,

20 and chips), optical media (e.g., CD ROM), information distributed by internet sites, and by RAM. The three-dimensional structural model of the channel protein is then generated by the interaction of the amino acid sequence and the computer system, using software known to those of skill in the art. The resulting three-dimensional computer model can then be saved on a computer readable substrate.

25 The amino acid sequence represents a primary structure that encodes the information necessary to form the secondary, tertiary and quaternary structure of the monomer and the heteromeric potassium channel protein comprising four monomers. The software looks at certain parameters encoded by the primary sequence to generate the structural model. These parameters are referred to as "energy terms," or anisotropic

30 terms and primarily include electrostatic potentials, hydrophobic potentials, solvent accessible surfaces, and hydrogen bonding. Secondary energy terms include van der Waals potentials. Biological molecules form the structures that minimize the energy

terms in a cumulative fashion. The computer program is therefore using these terms encoded by the primary structure or amino acid sequence to create the secondary structural model.

The tertiary structure of the protein encoded by the secondary structure is then formed on the basis of the energy terms of the secondary structure. The user at this point can enter additional variables such as whether the protein is membrane bound or soluble, its location in the body, and its cellular location, e.g., cytoplasmic, surface, or nuclear. These variables along with the energy terms of the secondary structure are used to form the model of the tertiary structure. In modeling the tertiary structure, the computer program matches hydrophobic faces of secondary structure with like, and hydrophilic faces of secondary structure with like.

Once the structure has been generated, potential ligand binding regions are identified by the computer system. Three-dimensional structures for potential ligands are generated by entering amino acid or nucleotide sequences or chemical formulas of compounds, as described above. The three-dimensional structure of the potential ligand is then compared to that of Kv6.2 protein to identify ligands that bind to Kv6.2. Binding affinity between the protein and ligands is determined using energy terms to determine which ligands have an enhanced probability of binding to the protein.

Computer systems are also used to screen for mutations, polymorphic variants, alleles and interspecies homologs of Kv6.2 genes. Such mutations can be associated with disease states. Once the variants are identified, diagnostic assays can be used to identify patients having such mutated genes associated with disease states. Identification of the mutated Kv6.2 genes involves receiving input of a first nucleic acid, e.g., SEQ ID NO:2 or SEQ ID NO:18, or an amino acid sequence encoding Kv6.2, selected from the group consisting of SEQ ID NO:1 or SEQ ID NO:17, and a conservatively modified versions thereof. The sequence is entered into the computer system as described above. The first nucleic acid or amino acid sequence is then compared to a second nucleic acid or amino acid sequence that has substantial identity to the first sequence. The second sequence is entered into the computer system in the manner described above. Once the first and second sequences are compared, nucleotide or amino acid differences between the sequences are identified. Such sequences can represent allelic differences in Kv6.2 genes, and mutations associated with disease states.

The first and second sequences described above can be saved on a computer readable substrate.

Kv6.2 monomers and the potassium channels containing these Kv6.2 monomers can be used with high density oligonucleotide array technology (e.g.,

5   GeneChip™) to identify homologs and polymorphic variants of Kv6.2 in this invention. In the case where the homologs being identified are linked to a known disease, they can be used with GeneChip™ as a diagnostic tool in detecting the disease in a biological sample, *see, e.g.*, Gunthand *et al., AIDS Res. Hum. Retroviruses* 14: 869-876 (1998); Kozal *et al., Nat. Med.* 2:753-759 (1996); Matson *et al., Anal. Biochem.* 224:110-106

10   (1995); Lockhart *et al., Nat. Biotechnol.* 14:1675-1680 (1996); Gingeras *et al., Genome Res.* 8:435-448 (1998); Hacia *et al., Nucleic Acids Res.* 26:3865-3866 (1998).

VIII. CELLULAR TRANSFECTION AND GENE THERAPY

The present invention provides the nucleic acids of Kv6.2 for the

15   transfection of cells *in vitro* and *in vivo*. These nucleic acids can be inserted into any of a number of well-known vectors for the transfection of target cells and organisms as described below. The nucleic acids are transfected into cells, *ex vivo* or *in vivo*, through the interaction of the vector and the target cell. The nucleic acid for Kv6.2, under the control of a promoter, then expresses a Kv6.2 monomer of the present invention, thereby

20   mitigating the effects of absent, partial inactivation, or abnormal expression of the Kv6.2 gene.

Such gene therapy procedures have been used to correct acquired and inherited genetic defects, cancer, and viral infection in a number of contexts. The ability to express artificial genes in humans facilitates the prevention and/or cure of many

25   important human diseases, including many diseases which are not amenable to treatment by other therapies (for a review of gene therapy procedures, *see* Anderson, *Science* 256:808-813 (1992); Nabel & Felgner, TIBTECH 11:211-217 (1993); Mitani & Caskey, TIBTECH 11:162-166 (1993); Mulligan, *Science* 926-932 (1993); Dillon, TIBTECH 11:167-175 (1993); Miller, *Nature* 357:455-460 (1992); Van Brunt, *Biotechnology*

30   6(10):1149-1154 (1998); Vigne, *Restorative Neurology and Neuroscience* 8:35-36 (1995); Kremer & Perricaudet, *British Medical Bulletin* 51(1):31-44 (1995); Haddada *et*

al., in *Current Topics in Microbiology and Immunology* (Doerfler & Böhm eds., 1995); and Yu *et al., Gene Therapy* 1:13-26 (1994)).

Delivery of the gene or genetic material into the cell is the first critical step in gene therapy treatment of disease. A large number of delivery methods are well known to those of skill in the art. Preferably, the nucleic acids are administered for *in vivo* or *ex vivo* gene therapy uses. Non-viral vector delivery systems include DNA plasmids, naked nucleic acid, and nucleic acid complexed with a delivery vehicle such as a liposome. Viral vector delivery systems include DNA and RNA viruses, which have either episomal or integrated genomes after delivery to the cell.

Methods of non-viral delivery of nucleic acids include lipofection, microinjection, biolistics, virosomes, liposomes, immunoliposomes, polycation or lipid:nucleic acid conjugates, naked DNA, artificial virions, and agent-enhanced uptake of DNA. Lipofection is described in, e.g., US Pat. No. 5,049,386, US Pat No. 4,946,787; and US Pat. No. 4,897,355 and lipofection reagents are sold commercially (e.g., Transfectam™ and Lipofectin™). Cationic and neutral lipids that are suitable for efficient receptor-recognition lipofection of polynucleotides include those of Felgner, WO 91/17424, WO 91/16024. Delivery can be to cells (*ex vivo* administration) or target tissues (*in vivo* administration).

The preparation of lipid:nucleic acid complexes, including targeted liposomes such as immunolipid complexes, is well known to one of skill in the art (*see, e.g.*, Crystal, *Science* 270:404-410 (1995); Blaese *et al., Cancer Gene Ther.* 2:291-297 (1995); Behr *et al., Bioconjugate Chem.* 5:382-389 (1994); Remy *et al., Bioconjugate Chem.* 5:647-654 (1994); Gao *et al., Gene Therapy* 2:710-722 (1995); Ahmad *et al., Cancer Res.* 52:4817-4820 (1992); U.S. Pat. Nos. 4,186,183, 4,217,344, 4,235,871, 4,261,975, 4,485,054, 4,501,728, 4,774,085, 4,837,028, and 4,946,787).

The use of RNA or DNA viral based systems for the delivery of nucleic acids take advantage of highly evolved processes for targeting a virus to specific cells in the body and trafficking the viral payload to the nucleus. Viral vectors can be administered directly to patients (*in vivo*) or they can be used to treat cells *in vitro* and the modified cells are administered to patients (*ex vivo*). Conventional viral based systems for the delivery of nucleic acids could include retroviral, lentivirus, adenoviral, adeno-

associated and herpes simplex virus vectors for gene transfer. Viral vectors are currently the most efficient and versatile method of gene transfer in target cells and tissues. Integration in the host genome is possible with the retrovirus, lentivirus, and adeno-associated virus gene transfer methods, often resulting in long term expression of the

5   inserted transgene. Additionally, high transduction efficiencies have been observed in many different cell types and target tissues.

The tropism of a retrovirus can be altered by incorporating foreign envelope proteins, expanding the potential target population of target cells. Lentiviral vectors are retroviral vector that are able to transduce or infect non-dividing cells and

10   typically produce high viral titers. Selection of a retroviral gene transfer system would therefore depend on the target tissue. Retroviral vectors are comprised of *cis*-acting long terminal repeats with packaging capacity for up to 6-10 kb of foreign sequence. The minimum *cis*-acting LTRs are sufficient for replication and packaging of the vectors, which are then used to integrate the therapeutic gene into the target cell to provide

15   permanent transgene expression. Widely used retroviral vectors include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), simian immunodeficiency virus (SIV), human immunodeficiency virus (HIV), and combinations thereof (*see, e.g.,* Buchscher *et al., J. Virol.* 66:2731-2739 (1992); Johann *et al., J. Virol.* 66:1635-1640 (1992); Sommerfelt *et al., Virol.* 176:58-59 (1990); Wilson *et al., J. Virol.*

20   63:2374-2378 (1989); Miller *et al., J. Virol.* 65:2220-2224 (1991); PCT/US94/05700).

In applications where transient expression of the nucleic acid is preferred, adenoviral based systems are typically used. Adenoviral based vectors are capable of very high transduction efficiency in many cell types and do not require cell division. With such vectors, high titer and levels of expression have been obtained. This vector

25   can be produced in large quantities in a relatively simple system. Adeno-associated virus ("AAV") vectors are also used to transduce cells with target nucleic acids, e.g., in the *in vitro* production of nucleic acids and peptides, and for *in vivo* and *ex vivo* gene therapy procedures (*see, e.g.,* West *et al., Virology* 160:38-47 (1987); U.S. Patent No. 4,797,368; WO 93/24641; Kotin, *Human Gene Therapy* 5:793-801 (1994); Muzyczka, *J. Clin.*

30   *Invest.* 94:1351 (1994)). Construction of recombinant AAV vectors are described in a number of publications, including U.S. Pat. No. 5,173,414; Tratschin *et al., Mol. Cell. Biol.* 5:3251-3260 (1985); Tratschin *et al., Mol. Cell. Biol.* 4:2072-2081 (1984);

Hermonat & Muzyczka, *Proc. Natl. Acad. Sci. U.S.A.* 81:6466-6470 (1984); and

Samulski *et al., J. Virol.* 63:03822-3828 (1989).

In particular, at least six viral vector approaches are currently available for

gene transfer in clinical trials, with retroviral vectors by far the most frequently used

5   system. All of these viral vectors utilize approaches that involve complementation of

defective vectors by genes inserted into helper cell lines to generate the transducing agent.

pLASN and MFG-S are examples are retroviral vectors that have been

used in clinical trials (Dunbar *et al., Blood* 85:3048-305 (1995); Kohn *et al., Nat. Med.*

1:1017-102 (1995); Malech *et al., Proc. Natl. Acad. Sci. U.S.A.* 94:22 12133-12138

10   (1997)). PA317/pLASN was the first therapeutic vector used in a gene therapy trial.

(Blaese *et al., Science* 270:475-480 (1995)). Transduction efficiencies of 50% or greater

have been observed for MFG-S packaged vectors (Ellem *et al., Immunol Immunother.*

44(1):10-20 (1997); Dranoff *et al., Hum. Gene Ther.* 1:111-2 (1997)).

Recombinant adeno-associated virus vectors (rAAV) are a promising

15   alternative gene delivery systems based on the defective and nonpathogenic parvovirus

adeno-associated type 2 virus. All vectors are derived from a plasmid that retains only

the AAV 145 bp inverted terminal repeats flanking the transgene expression cassette.

Efficient gene transfer and stable transgene delivery due to integration into the genomes

of the transduced cell are key features for this vector system (Wagner *et al., Lancet*

20   351:9117 1702-3 (1998), Kearns *et al., Gene Ther.* 9:748-55 (1996)).

Replication-deficient recombinant adenoviral vectors (Ad) are

predominantly used transient expression gene therapy, because they can be produced at

high titer and they readily infect a number of different cell types. Most adenovirus

vectors are engineered such that a transgene replaces the Ad E1a, E1b, and E3 genes;

25   subsequently the replication defector vector is propagated in human 293 cells that supply

deleted gene function in trans. Ad vectors can transduce multiple types of tissues *in vivo*,

including nondividing, differentiated cells such as those found in the liver, kidney and

muscle system tissues. Conventional Ad vectors have a large carrying capacity. An

example of the use of an Ad vector in a clinical trial involved polynucleotide therapy for

30   antitumor immunization with intramuscular injection (Sterman *et al., Hum. Gene Ther.*

7:1083-9 (1998)). Additional examples of the use of adenovirus vectors for gene transfer

in clinical trials include Rosenecker *et al., Infection* 241:5-10 (1996); Sterman *et al.,*

Hum. Gene Ther. 9:7 1083-1089 (1998); Welsh *et al., Hum. Gene Ther.* 2:205-18 (1995); Alvarez *et al., Hum. Gene Ther.* 5:597-613 (1997); Topf *et al., Gene Ther.* 5:507-513 (1998); Sterman *et al., Hum. Gene Ther.* 7:1083-1089 (1998).

5   Packaging cells are used to form virus particles that are capable of infecting a host cell. Such cells include 293 cells, which package adenovirus, and ψ2 cells or PA317 cells, which package retrovirus. Viral vectors used in gene therapy are usually generated by producer cell line that packages a nucleic acid vector into a viral particle. The vectors typically contain the minimal viral sequences required for packaging and subsequent integration into a host, other viral sequences being replaced by

10   an expression cassette for the protein to be expressed. The missing viral functions are supplied in *trans* by the packaging cell line. For example, AAV vectors used in gene therapy typically only possess ITR sequences from the AAV genome which are required for packaging and integration into the host genome. Viral DNA is packaged in a cell line, which contains a helper plasmid encoding the other AAV genes, namely *rep* and *cap*, but

15   lacking ITR sequences. The cell line is also infected with adenovirus as a helper. The helper virus promotes replication of the AAV vector and expression of AAV genes from the helper plasmid. The helper plasmid is not packaged in significant amounts due to a lack of ITR sequences. Contamination with adenovirus can be reduced by, e.g., heat treatment to which adenovirus is more sensitive than AAV.

20   In many gene therapy applications, it is desirable that the gene therapy vector be delivered with a high degree of specificity to a particular tissue type. A viral vector is typically modified to have specificity for a given cell type by expressing a ligand as a fusion protein with a viral coat protein on the viruses outer surface. The ligand is chosen to have affinity for a receptor known to be present on the cell type of interest. For

25   example, Han *et al., Proc. Natl. Acad. Sci. U.S.A.* 92:9747-9751 (1995), reported that Moloney murine leukemia virus can be modified to express human heregulin fused to gp70, and the recombinant virus infects certain human breast cancer cells expressing human epidermal growth factor receptor. This principle can be extended to other pairs of virus expressing a ligand fusion protein and target cell expressing a receptor. For

30   example, filamentous phage can be engineered to display antibody fragments (e.g., FAB or Fv) having specific binding affinity for virtually any chosen cellular receptor. Although the above description applies primarily to viral vectors, the same principles can

be applied to nonviral vectors. Such vectors can be engineered to contain specific uptake sequences thought to favor uptake by specific target cells.

Gene therapy vectors can be delivered *in vivo* by administration to an individual patient, typically by systemic administration (e.g., intravenous, intraperitoneal,

5 intramuscular, subdermal, or intracranial infusion) or topical application, as described below. Alternatively, vectors can be delivered to cells *ex vivo*, such as cells explanted from an individual patient (e.g., lymphocytes, bone marrow aspirates, tissue biopsy) or universal donor hematopoietic stem cells, followed by reimplantation of the cells into a patient, usually after selection for cells which have incorporated the vector.

10 *Ex vivo* cell transfection for diagnostics, research, or for gene therapy (e.g., via re-infusion of the transfected cells into the host organism) is well known to those of skill in the art. In a preferred embodiment, cells are isolated from the subject organism, transfected with a nucleic acid (gene or cDNA), and re-infused back into the subject organism (e.g., patient). Various cell types suitable for *ex vivo* transfection are well

15 known to those of skill in the art (*see, e.g.*, Freshney *et al., Culture of Animal Cells, A Manual of Basic Technique* (3rd ed. 1994)) and the references cited therein for a discussion of how to isolate and culture cells from patients).

In one embodiment, stem cells are used in *ex vivo* procedures for cell transfection and gene therapy. The advantage to using stem cells is that they can be

20 differentiated into other cell types *in vitro*, or can be introduced into a mammal (such as the donor of the cells) where they will engraft in the bone marrow. Methods for differentiating CD34+ cells *in vitro* into clinically important immune cell types using cytokines such a GM-CSF, IFN-γ and TNF-α are known (*see* Inaba *et al., J. Exp. Med.* 176:1693-1702 (1992)).

25 Stem cells are isolated for transduction and differentiation using known methods. For example, stem cells are isolated from bone marrow cells by panning the bone marrow cells with antibodies which bind unwanted cells, such as CD4+ and CD8+ (T cells), CD45+ (panB cells), GR-1 (granulocytes), and Iad (differentiated antigen presenting cells) (*see* Inaba *et al., J. Exp. Med.* 176:1693-1702 (1992)).

30 Vectors (e.g., retroviruses, adenoviruses, liposomes, etc.) containing therapeutic nucleic acids can be also administered directly to the organism for transduction of cells *in vivo*. Alternatively, naked DNA can be administered.

Administration is by any of the routes normally used for introducing a molecule into ultimate contact with blood or tissue cells. Suitable methods of administering such nucleic acids are available and well known to those of skill in the art, and, although more than one route can be used to administer a particular composition, a particular route can

5   often provide a more immediate and more effective reaction than another route.

Administration is by any of the routes normally used for introducing a molecule into ultimate contact with blood or tissue cells. The nucleic acids are administered in any suitable manner, preferably with pharmaceutically acceptable carriers. Suitable methods of administering such nucleic acids are available and well

10   known to those of skill in the art, and, although more than one route can be used to administer a particular composition, a particular route can often provide a more immediate and more effective reaction than another route.

IX.   PHARMACEUTICAL COMPOSITIONS

15   Pharmaceutically acceptable carriers are determined in part by the particular composition being administered (e.g., nucleic acid, protein, modulatory compounds or transduced cell), as well as by the particular method used to administer the composition. Accordingly, there are a wide variety of suitable formulations of pharmaceutical compositions of the present invention (*see, e.g., Remington's*

20   *Pharmaceutical Sciences*, 17th ed., 1989).

Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the packaged nucleic acid suspended in diluents, such as water, saline or PEG 400; (b) capsules, sachets or tablets, each containing a predetermined amount of the active ingredient, as liquids, solids, granules or gelatin; (c)

25   suspensions in an appropriate liquid; and (d) suitable emulsions. Tablet forms can include one or more of lactose, sucrose, mannitol, sorbitol, calcium phosphates, corn starch, potato starch, microcrystalline cellulose, gelatin, colloidal silicon dioxide, talc, magnesium stearate, stearic acid, and other excipients, colorants, fillers, binders, diluents, buffering agents, moistening agents, preservatives, flavoring agents, dyes, disintegrating

30   agents, and pharmaceutically compatible carriers. Lozenge forms can comprise the active ingredient in a flavor, e.g., sucrose, as well as pastilles comprising the active ingredient in

an inert base, such as gelatin and glycerin or sucrose and acacia emulsions, gels, and the like containing, in addition to the active ingredient, carriers known in the art.

The compound of choice, alone or in combination with other suitable components, can be made into aerosol formulations (i.e., they can be "nebulized") to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

Suitable formulations for rectal administration include, for example, suppositories, which consist of the packaged nucleic acid with a suppository base. Suitable suppository bases include natural or synthetic triglycerides or paraffin hydrocarbons. In addition, it is also possible to use gelatin rectal capsules which consist of a combination of the compound of choice with a base, including, for example, liquid triglycerides, polyethylene glycols, and paraffin hydrocarbons.

Formulations suitable for parenteral administration, such as, for example, by intraarticular (in the joints), intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. In the practice of this invention, compositions can be administered, for example, by intravenous infusion, orally, topically, intraperitoneally, intravesically or intrathecally. Parenteral administration and intravenous administration are the preferred methods of administration. The formulations of commends can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials.

Injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. Cells transduced by nucleic acids for *ex vivo* therapy can also be administered intravenously or parenterally as described above.

The dose administered to a patient, in the context of the present invention should be sufficient to effect a beneficial therapeutic response in the patient over time. The dose will be determined by the efficacy of the particular vector employed and the condition of the patient, as well as the body weight or surface area of the patient to be treated. The size of the dose also will be determined by the existence, nature, and extent

of any adverse side-effects that accompany the administration of a particular vector, or transduced cell type in a particular patient.

In determining the effective amount of the vector to be administered in the treatment or prophylaxis of conditions owing to diminished or aberrant expression of the

5   Kv6.2 channels comprising a human Kv6.2 alpha subunit, the physician evaluates circulating plasma levels of the vector, vector toxicities, progression of the disease, and the production of anti-vector antibodies. In general, the dose equivalent of a naked nucleic acid from a vector is from about 1 µg to 100 µg for a typical 70 kilogram patient, and doses of vectors which include a retroviral particle are calculated to yield an

10   equivalent amount of therapeutic nucleic acid.

For administration, compounds and transduced cells of the present invention can be administered at a rate determined by the LD-50 of the inhibitor, vector, or transduced cell type, and the side-effects of the inhibitor, vector or cell type at various concentrations, as applied to the mass and overall health of the patient. Administration

15   can be accomplished via single or divided doses.

Transduced cells are prepared for reinfusion according to established methods (see, e.g., Abrahamsen et al., J. Clin. Apheresis 6:48--53 (1991); Carter et al., J. Clin. Apheresis 4:113-117 (1998); Aebersold et al., J. Immunol. Meth. 112:1-7 (1998); Muul et al., J. Immunol. Methods 101:171-181 (1987); and Carter et al., Transfusion

20   27:362-365 (1987)).

X.   KITS

Human Kv6.2 and its homologs are useful tools for examining expression and regulation of potassium channels. Human Kv6.2-specific reagents that specifically

25   hybridize to Kv6.2 nucleic acid, such as Kv6.2 probes and primers, and Kv6.2-specific reagents that specifically bind to the Kv6.2 protein, e.g., Kv6.2 antibodies are used to examine expression and regulation.

Nucleic acid assays for the presence of Kv6.2 DNA and RNA in a sample include numerous techniques are known to those skilled in the art, such as Southern

30   analysis, northern analysis, dot blots, RNase protection, S1 analysis, amplification techniques such as PCR and LCR, and in situ hybridization. In in situ hybridization, for example, the target nucleic acid is liberated from its cellular surroundings in such as to be

available for hybridization within the cell while preserving the cellular morphology for subsequent interpretation and analysis. The following articles provide an overview of the art of *in situ* hybridization: Singer *et al., Biotechniques* 4:230-250 (1986); Haase *et al., Methods in Virology*, vol. VII, pp. 189-226 (1984); and *Nucleic Acid Hybridization: A*

5   *Practical Approach* (Hames *et al.*, eds. 1987). In addition, Kv6.2 protein can be detected with the various immunoassay techniques described above. The test sample is typically compared to both a positive control (e.g., a sample expressing recombinant Kv6.2 monomers) and a negative control.

The present invention also provides for kits for screening modulators of

10 the heteromeric potassium channels. Such kits can be prepared from readily available materials and reagents. For example, such kits can comprise any one or more of the following materials: Kv6.2 monomers, reaction tubes, and instructions for testing the activities of potassium channels containing Kv6.2. A wide variety of kits and components can be prepared according to the present invention, depending upon the

15 intended user of the kit and the particular needs of the user. For example, the kit can be tailored for *in vitro* or *in vivo* assays for measuring the activity of a potassium channel comprising a Kv6.2 monomer.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application

20 were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit

25 or scope of the appended claims.

EXAMPLES

The following example is provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of noncritical

30 parameters that could be changed or modified to yield essentially similar results.

Example I: Cloning of mouse Kv6.2

Using PCR and primers, according to standard conditions, mouse Kv6.2 is amplified from whole brain cDNA. The following primers are used for amplification: ATGCCCATGTCTTCCAGAGACAGG (SEQ ID NO:3), and

5 GATGTCTAGAGGGAGTTACATGTAGCG (SEQ ID NO:4).

The cDNA is prepared from total RNA isolated from whole brain according to standard methods. Kv6.2 is amplified with the primers described above using the following conditions: 15 seconds at 96°C, 15 seconds at 72-60°C, and 3 minutes at 72°C for 40 cycles.

10 The PCR products are subcloned into plasmids and sequenced according to standard techniques. The nucleotide and amino acid sequences of mouse Kv6.2 are provided, respectively, in SEQ ID NO:2 and SEQ ID NO:1 (see also Figure 1 for a comparison of human and mouse Kv6.2 amino acid sequences).

15 Example II: Cloning of human Kv6.2

An approximately 200 bp fragment of the human Kv6.2 gene was cloned using RT-PCR from human whole brain mRNA with a sense primer from the pore region of mouse Kv6.2 (TAGCATCCCGGCATCCTATTGGTG; SEQ ID NO:11) and a degenerate antisense primer to the S6 region (AGGAGTGAGAGAACGTRTGRAADAT;

20 SEQ ID NO:12). Cycling conditions used were 20 cycles of 95 degrees - 15 seconds, 65-45 degrees – 15 seconds (1 degree dropped/cycle), 72 degrees 2 minutes, followed by 20 cycles of 95 degrees – 15 seconds, 45 degrees – 15 seconds, 72 degrees – 2 minutes.

The 3' end of the gene was cloned using a single round of standard 3' RACE PCR using the gene specific sense primer binding to the P region

25 (CATCCTATTGGTGGGCCATCATCT; SEQ ID NO:13). Cycling conditions were: 24 cycles of 95 degrees - 15 seconds, 72-60 degrees - 15 seconds (0.5 degrees dropped per cycle), 72 degrees - 2 minutes, followed by 21 cycles of 95 degrees-15 seconds, 60 degrees-15 seconds, 72 degrees-2 minutes. A single band of approximately 500 bp was isolated and sequenced. It contained the P region through the stop codon of human

30 Kv6.2.

The 5' end was cloned with two rounds of 5' RACE PCR using nested gene specific oligos. The 1st round reaction conditions were identical to that used for the 3' RACE, except that a different gene specific primer (GGGAGAAGGTGTGGAAGATAGACG; SEQ ID NO:10) which binds to S6 in the

5   antisense direction was used. One tenth of a microliter of this reaction was used as a template for a second reaction in which the a nested gene specific antisense primer was used (GCCACCATCTGGCCTGGCACACTG; SEQ ID NO:14). The cycling conditions for this reaction were 95 degrees – 15 seconds, 60 degrees 15 seconds, 72 degrees 2 minutes (25 cycles). A single band of approximately 1.7 Kb was isolated. It was found

10   to contain the 5' end of the human Kv6.2 gene, including the initiator methionine through the P region.

        The entire human Kv6.2 gene was then amplified in a single piece for an expression vector the sense primer TCTTGAATTCCGCCATGCCCATGCCTTCCAGAGACGG (SEQ ID NO:15) (which

15   adds an EcoR1 restriction site and Kozak consensus to the initiator methionine) and the antisense primer CTGGGCTCTAGAAACACCACCAGGT (SEQ ID NO:16), which lies in the 3' UTR of human Kv6.2. Cycles were identical to that used for the 3' RACE PCR. A single band of approximately 1.7 Kb was isolated and found to contain the entire open reading frame of human Kv6.2. the primer pair ATGCCCATGCCTTCCAGAGACGG

20   (SEQ ID NO:7) and TTACATGTGCATGATAGGCAAGGCTG(SEQ ID NO:8) is sufficient to amplify the open reading frame of Kv6.2 under these conditions. The nucleotide and amino acid sequences of mouse Kv6.2 are provided, respectively, in SEQ ID NO:18 and SEQ ID NO:17 (*see also* Figure 1 for a comparison of human and mouse Kv6.2 amino acids sequences).

25

Example III: Expression and voltage-gated activity of heteromeric channels containing Kv6.2 monomers

        Human Kv6.2 monomer was co-r.pressed in *Xenopus* oocytes with human Kv2.1 monomer according to standard methodology, to demonstrate its ability to

30   form heteromeric potassium channels with voltage-gated activity (*see* Figure 2). Mouse Kv6.2 and human Kv2.1 were also co-expressed. Changes in current magnitude are

indirectly measured using a reporter voltage-sensitive fluorescent dye (*see, e.g.*, Etts *et al.*, *Chemistry and Physiology of Lipids*, 69:137 (1994)). Changes in current magnitude are also measured directly using electrophysiology, and with ion flux. Kv6.2 expressed alone was electrically silent (*see* Figure 2).

SEQUENCE LISTING

Mouse Kv6.2 amino acid sequence (SEQ ID NO:1)

MPMSSRDRDLHPGHHHFGSCSPLSQLWPGPEPKSVKGLYYSRARKVGNQD

5   ASPEANLKEILVNVGGQRYLLPWSTLDAFPLSRLSRLRLCRSHEEITQLC

DDYDEDSQEFFFDRNPSAFGVIVSFLAAGKLVLLREMCALSFREELSYWG

IEETNLERCCLRKLLKKLEEAAELRREEAAQRQQQRQACHSEVQASRWAR

SMNQLREMVEDPQSGLPGKVFACLSVLFVATTAVSLCVSTMPDFRAEEGK

GECTRKCYYIFVVESICVAWFSLEFCLRFVQAPNKCQFFRGPLNVIDILA

10   ISPYYVSLAVSDESPEAGERPSSSSYLEKVGLVLRVLRALRILYVMRLAR

HSLGLQTLGLTVRRCAREFGLLMLFLAVAVTLFSPLVYVAENESGRVLEF

TSIPASYWWAIISMTTVGYGDMVPRSVPGQMVALSSILSGILIMAFPATS

IFHTFSHSYLKV6.2REQEQVQARLRRLQNTNSASERELLSDVDDLVPEG

LTSPGRYM

15

Mouse Kv6.2 nucleotide sequence (SEQ ID NO:2)

ATGCCCATGTCTTCCAGAGACAGGGACTTGCATCCTGGACACCATCACTT

TGGCTCCTGCAGCCCCTTGAGCCAGCTCTGGCCGGGCCCCGAGCCTAAGT

CAGTCAAGGGCCTTTACTACAGCAGGGCCCGGAAGGTGGGCAACCAGGAC

20   GCCTCTCCGGAGGCCAACTTGAAGGAGATCCTAGTGAATGTGGGTGGCCA

GCGGTACCTGCTGCCCTGGAGCACCCTGGATGCCTTCCCGCTGAGCCGCC

TGAGCAGGCTCCGGCTGTGCCGCAGCCATGAGGAGATCACGCAGCTCTGC

GATGACTACGATGAGGACAGCCAGGAGTTCTTCTTCGACAGGAACCCCAG

CGCCTTCGGGGTGATCGTGAGCTTCCTGGCCGCGGGAAAGCTGGTGCTTC

25   TGCGAGAGATGTGCGCCCTGTCCTTCCGGGAGGAGCTGAGCTACTGGGGC

ATCGAGGAAACCAACCTGGAGCGCTGCTGCCTGCGCAAGCTGCTGAAGAA

GCTGGAGGAGGCGGCCGAGCTGCGCCGGGAGGAGGCTGCCCAGCGCCAGC

AGCAGCCCCAGGCCTGCCACTCCGAGGTGCAGGCTTCACGATGGGCCCGC

AGCATGACCAGCTGCGTGAAATGGTGGAGGACCCACAGTCGGGGCTGCC

30   CGGGAAGGTCTTCGCCTGCCTCTCCGTGCTCTTCGTGGCAACCACGGCTG

TCAGCCTGTGTGTGAGCACCATGCCCGACTTCAGGGCTGAGGAGGGCAAG

GGAGAATGCACTAGAAAGTGCTATTACATCTTCGTGGTGGAATCCATCTG

TGTGGCCTGGTTCTCGCTGGAGTTTTGCCTGCGCTTTGTCCAGGCCCCGA
ACAAATGTCAGTTCTTCCGCGGGCCCCTGAATGTCATCGACATTCTAGCC
ATCTCCCCATACTATGTGTCGCTCGCAGTGTCTGACGAATCCCCGGAGGC
AGGCGAGAGGCCGAGCAGCAGCTCCTACCTGGAGAAAGTGGGGTTAGTGC
5 TGCGTGTTCTGCGGGCACTACGCATCCTCTACGTAATGCGCCTGGCTCGC
CACTCCCTGGGGCTGCAGACGCTGGGCCTCACTGTGCGCCGCTGCGCCCG
AGAGTTTGGTCTCCTGATGCTCTTCCTGGCTGTGGCGGTTACCCTCTTCT
CACCGTTGGTCTATGTAGCTGAGAATGAGTCCGGAAGGGTCCTGGAGTTC
ACTAGCATCCCCGCATCCTATTGGTGGGCCATCATCTCCATGACGACCGT
10 GGGCTATGGGGACATGGTCCCTCGCAGCGTCCCGGACAGATGGTGGCTC
TGAGCAGCATCCTTAGCGGGATCCTTATCATGGCTTTCCCAGCCACATCC
ATCTTCCACACGTTCTCTCACTCCTACCTGGAGCTGAAGCGGGAGCAGGA
GCAGGTCCAGGCCCGCCTCCGGCGTCTTCAGAACACCAATTCGGCCAGCG
AACGGGAGCTCCTGAGTGACGTAGATGATCTGGTCCCTGAGGGTCTGACC
15 TCCCCAGGCCGCTACATG

Human Kv6.2 amino acid sequence (SEQ ID NO:17)

MPMPSRDGGLHPRHHHYGSHSPWSQLLSSPMETPSIKGLYYRRVRKVGALDASPVDLKKE
ILINVGGRRYLLPWSTLDRFPLSRLSKLRLCRSYEEIVQLCDDYDEDSQEFFFDRSPSAF
20 GVIVSFLAAGKLVLLQEMCALSFQEELAYWGIEEAHLERCCLRKLLRKLEELEELAKLHR
EDVLRQQRETRRPASHSSRWGLCMNRLREMVENPQSGLPGKVFACLSILFVATTAVSLCV
STMPDLRAEEDQGECSRKCYYIFIVETICVAWFSLEFCLRFVQAQDKCQFFQGPLNIIDI
LAISPYYVSLAVSEEPPEDGERPSRSSYLEKVGLVLRVLRALRILYVMRLARHSLGLQTL
GLTVRRCTCEFGLLLLFLAVAITLFSPLVYVAEKESGRVLEFTSIPASYWWAIISMTTVG
·25 YGDMVPRSVPGQMVALSSILSGILIMAFPATSIFHTFSHSYLKV6.2KEQEQLQARLRHL
QNTGPASECELLDPHVASEHELMNDVNDLILEGPALPIMHM

Human Kv6.2 nucleotide sequence (SEQ ID NO:18)

CTTCCCCTTCATCTCCACCAGAA.CCTGTCCCTTCCCTGGGCACCAAGAGATGGGCTCCC
30 CTTGCCTGGCAGAGAAACAGCTGGAAACTGGCTCCCTGAGACAAGAAGACTGGTAAACCC
AGCGCTTCCTACCTGGTGGTCTTCAGCAATGCCCATGCCTTCCAGAGACGGGGGCCTGCA
TCCCAGACACCACCACTATGGTTCCCACAGCCCTTGGAGTCAGCTCCTGTCCAGCCCCAT

GGAGACGCCGTCCATCAAGGGCCTTTACTACCGGAGGGTGCGGAAGGTGGGTGCCCTGGA

CGCCTCCCCAGTGGACCTGAAGAAGGAGATCCTGATCAACGTGGGGGGCAGGAGGTATCT

CCTCCCCTGGAGCACACTGGACCGGTTCCCGCTGAGCCGCCTGAGCAAACTCAGGCTCTG

TCGGAGCTACGAGGAGATCGTGCAGCTCTGCGATGATTACGACGAGGACAGCCAGGAGTT

5   CTTCTTCGACAGGAGCCCCAGCGCCTTCGGGGTGATCGTGAGCTTCCTGGCGGCCGGGAA

GCTGGTGCTTCTGCAGGAGATGTGCGCGCTGTCCTTCCAGGAGGAGCTGGCCTACTGGGG

CATCGAGGAGGCCCACCTGGAGAGGTGCTGCCTGCGGAAGCTGCTGAGGAAGCTGGAGGA

GCTGGAGGAGCTGGCCAAGCTGCACAGGGAGGACGTACTGAGGCAGCAGAGGGAGACCCG

CCGCCCCGCCTCGCACTCCTCGCGCTGGGGCCTGTGCATGAACCGGCTGCGCGAGATGGT

10 GGAAAACCCGCAGTCCGGGCTGCCCGGGAAGGTCTTCGCTTGCCTCTCCATCCTCTTCGT

GGCCACCACAGCCGTCAGCCTGTGTGTCAGCACCATGCCCGACCTCAGGGCAGAGGAGGA

CCAGGGCGAATGCTCTCGGAAGTGCTACTATATTTTCATCGTGGAGACCATCTGCGTGGC

CTGGTTCTCCCTGGAGTTCTGCCTGCGGTTTGTCCAGGCCCAAGACAAGTGTCAGTTCTT

CCAGGGGCCCCTGAACATCATCGACATCCTGGCCATCTCCCCATACTACGTGTCGCTGGC

15 GGTGTCTGAGGAGCCCCCGGAGGACGGCGAGAGGCCGAGCAGGAGCTCCTACCTGGAGAA

GGTGGGGCTGGTCCTGCGTGTGCTGCGAGCGCTGCGCATCCTCTACGTGATGCGCCTGGC

TCGCCACTCGCTGGGGCTGCAGACGCTGGGGCTCACCGTGCGCCGTTGCACATGTGAGTT

CGGCCTGCTCCTTCTCTTCCTGGCCGTGGCCATCACCCTCTTCTCCCCTTTGGTCTACGT

GGCCGAGAAGGAGTCCGGGCGGGTGCTGGAGTTCACCAGCATCCCCGCCTCCTATTGGTG

20 GGCCATCATCTCCATGACAACGGTGGGCTACGGGACATGGTGCCCCGCAGTGTGCCAGG

CCAGATGGTGGCCCTCAGCAGCATCCTGAGCGGGATCCTCATCATGGCCTTCCCGGCCAC

GTCTATCTTCCACACCTTCTCCCACTCCTACCTGGAGCTCAAGAAGGAGCAGGAGCAGCT

TCAGGCCCGCCTCCGCCACCTCCAAAACACCGGTCCAGCCAGTGAATGTGAACTCCTGGA

CCCCCATGTGGCCAGTGAACATGAGCTCATGAACGATGTCAATGACCTAATCCTGGAGGG

25 CCCAGCCTTGCCTATCATGCACATGTAACTCAGCACCCCCATGACTACATGGTAACCTC

AACCCATCACCCTGCCTGAAACACACTCAAGGGTACCCGCATAGACCACCTGGTGGTGT

TTCTAGAGCCCAGGGAAGACTTTCAAAGCTGGAGGGGCATAAGGCCACAGAGGCTGTGTG

TCTGTGATCCTTGTCCCTCGGGGCCCCGATGTCCCAGGCTGACTGTGTCCAGCCTGCTTG

CCTTTTCCTCTCTCTGCCCATCTACTGAGCATGTCCAATC.TGCTGGAGTAGCTCAGTCT

30 CCTTTCATTCTCTTTTCCTTCCCAGCAGAGGCTTTAACATCC